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The Journal of Allergy

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American Academy of Allergy

Third Annual Meeting, New York, N. Y., Nov. 25 to 27, 1946

(Continued)

CHARCOT-LEYDEN CRYSTALS

A STUDY OF THE CONDITIONS NECESSARY FOR THEIR FORMATION

MAX SAMTER, M.D.,*† CHICAGO, ILL.

CHARCOT and Robin,[1] in 1853, described peculiar crystals which they had found in the cardiac blood and the spleen of a leucemic patient. Leyden,[2] in 1872, recognized the same crystals in asthmatic sputum. They appear as slender needles of uniform shape—two hexagonal pyramids joined base to base—of various size reaching a maximum length of 75 to 100 microns which have since been known as Charcot-Leyden crystals.

Numerous observations have established the relation between the presence of disintegrating eosinophilic leucocytes and Charcot-Leyden crystals (Schwarz,[3] Taratymow,[4] Acton,[5] Wätjen,[6] Henschen,[7] Bahrmann,[8] Fingerland,[9] Thomson,[10] Turner, Jackson, and Parker,[11] Thompson and Paddock[12]). No essential information has been added, however, since Askanazy's[13] summary in 1927 in which he stated "we have to conclude that intimate chemical relations exist between eosinophilic myelocytes and leucocytes and Charcot-Leyden crystals, but we have no grounds to assume the chemical identity between oxyphilic granules and Charcot-Leyden crystals."

The history of the chemical investigation of Charcot-Leyden crystals presents a fascinating controversy. Böttcher[14] and Schreiner[15] isolated crystals out of human sperm and prostatic secretion which they held to be Charcot-Leyden crystals. Although Cohn[16] pointed out as early as 1895 obvious differences between the two formations, the notion of their identity has never disappeared from the literature. Wrede, Boldt, and Buch[17] maintain that Charcot-Leyden crystals consist of spermin phosphate $C_{10}H_{26}N_4 \cdot 2H_3PO_4 + 6\,H_2O$ and confirm their belief by the determination of spermin in normal and leucemic spleen—a concept which has been adopted with reservations by Neumann in 1927,[18] by Henschen in 1934.[7]

*Read at the Third Annual Meeting of the American Academy of Allergy, New York, Nov. 27, 1946.

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Liebreich,[19] in 1921, used a different approach. He experimented on a mixture of normal human blood, sodium citrate, and gum acacia, and observed during the phase of "incomplete coagulation" in the centrifuged specimen the transitory appearance of numerous Charcot-Leyden crystals in the gelatinous layer of plasma which was in contact with the buffy coat. The complexity of his experiments, as well as a number of disputed statements and erroneous conclusions on the artificial production of eosinophiles, have discredited his research in spite of the fact that his observations on the formation of Charcot-Leyden crystals have been shown to be accurate and represent an important contribution for the understanding of their formation. His interpretation, however, that their mother substance is fibrin, had to be discarded.

Storm van Leeuwen and Nijk,[20] in 1923, published a preliminary report that they had succeeded in producing Charcot-Leyden crystals in vitro by adding a 10 per cent solution of disodiumphosphate to a 10 per cent solution of calcium chloride: $Na_2H\ PO_4 + CaCl = CaHPO_4 + 2NaCl$. Their conclusion that Charcot-Leyden crystals are crystals of labile secondary calcium phosphate has been contested by several investigators, but Bogen[21] confirmed, in careful polarization studies, their identical crystallographic behavior.

Chemical studies of Charcot-Leyden crystals in situ have been repeatedly attempted. The results are contradictory, due to the minute amount and the lack of uniformity of available material. The crystals represent end products of cellular metabolism. Separation of the crystals will facilitate their final chemical analysis.

It seemed necessary, therefore, in view of the variations of technique used by previous observers, to investigate the conditions necessary for their formation in standardized preparations.

I. STUDIES ON INDIVIDUAL EOSINOPHILIC LEUCOCYTES

A. *Unstained Preparations.*—

Experimental problem: In view of the known fact that eosinophilic leucocytes—referred to in this study as "eosinophiles"—are, in a majority of instances, part of the process which leads to the development of Charcot-Leyden crystals, it seemed necessary to observe isolated eosinophiles during crystal formation.

Experimental procedure: The study was carried out on blood obtained from patients who had varying absolute eosinophile counts. The counts ranged from 0 to 4,400 per cubic millimeter of blood determined by Zappert's[22] hematocytometric method recently reviewed and reintroduced by Discombe.[23] The specimens examined included normal individuals, 8; patients with familial eosinophilia, 1; with allergic diseases, 18; with parasites, 4; with skin disorders, 6; and with leucemia, 4. The total number of preparations in this group was 210.

One drop of blood was taken from the patient's finger tip, covered with a cover slip, sealed with petrolatum, and kept at room temperature. The technique of the initial experiments followed the procedure outlined by Thompson and Paddock.[12] The period of observation, however, was extended to fourteen days.

Results: Thompson and Paddock had found that the occurrence of Charcot-Leyden crystals in their preparations depended on a high absolute eosinophile count at the site of formation. The minimum concentration mentioned was 3,200 eosinophiles per cubic millimeter in a case of trichinosis. Our own observations convinced us that while the conclusions reached by Thompson and Paddock are correct in principle, formation of Charcot-Leyden crystals is possible at levels which are much lower than those quoted by these authors. We were fortunate enough to observe a patient with dermatomyositis who entered the hospital with an absolute eosinophile count of 3,280 eosinophiles per cubic millimeter. At this time, a considerable number of Charcot-Leyden crystals formed in the preparations: he was "C-L positive." (This term will be used throughout this paper as follows: studies in which Charcot-Leyden crystals were found to develop are termed C-L positive; preparations which failed to develop are called C-L negative.) Under x-ray treatment this patient's total white cell count dropped to 2,800 leucocytes per cubic millimeter, his absolute eosinophile count to 40 eosinophiles per cubic millimeter: he became C-L negative. Formation of Charcot-Leyden crystals was resumed, however, after his total white count was restored to 8,400 leucocytes per cubic millimeter, his absolute eosinophile count to 960 eosinophiles per cubic millimeter.

It was ascertained, moreover, that each individual Charcot-Leyden crystal originated from a single eosinophile. In a number of cases, two or three Charcot-Leyden crystals developed from one eosinophile. Intracellular formation of the crystals was seen from the second day on, with the tip of the crystal piercing the cellular membrane. It was common to find a considerable number of paracellular crystals, adjoining the outer surface of the cellular membrane during the third and fourth day, with a gradual increase of free, unattached crystals during the following days. Even in specimens with a high absolute eosinophile count, only a small minority of eosinophiles produced Charcot-Leyden crystals.

B. *Stained Preparations.*—

Experimental problem: In order to obtain additional information about the development of Charcot-Leyden crystals in individual eosinophiles, we attempted to observe their development in supravital-stained preparations.

Experimental procedure: With the exception of four patients with allergic diseases, the same individuals contributed blood for both unstained and stained preparations. The total number of preparations in the second group was 318. Two methods of staining were used concurrently on each specimen. The technique selected for supravital staining and subsequent observation of eosinophiles is in many respects similar to the one used in unstained specimens.

For the first method, one drop of the patient's blood is placed on the slide, mixed with one drop of a solution of dye in physiologic saline, covered with a cover slip, sealed with petrolatum, and transferred into a moistened Petri dish for incubation for one hour at 37° C. The preparation is then returned to room temperature and observed for a period of fourteen days in the same manner as the unstained preparations.

The second method follows the supravital technique described by Cunningham and Tompkins.[24] The slides are prepared prior to the application of blood: "after the slide has been flamed and cooled, it is flooded with the dilute stain; the excess stain is promptly drained back into the bottle and the slide allowed to dry." The cover slip, carrying the blood from the patient's finger, is inverted and placed upon the stained side of the slide. The specimen is sealed with petrolatum and incubated in order to insure a sufficiently active incorporation of dye into the cells. From then on, the procedure follows the routine outlined before.

Dyes used for the first method were: neutral red, 0.02 per cent solution in physiologic saline; Wright's stain, 0.01 per cent solution in physiologic saline; Congo-red, 0.01 per cent solution in physiologic saline; control, physiologic saline without addition of dye.

Dyes used for the second method were: neutral red, 0.02 per cent solution in absolute ethyl alcohol; Wright's stain, 0.01 per cent solution in absolute ethyl alcohol; Congo red, 0.01 per cent solution in absolute ethyl alcohol.

The toxicity of the dyes, which is of foremost importance for supravital study of cells, is of no significance for the present investigation which is concerned with postmortem changes.

Results:

1. Either of the two modifications produced brilliant supravital staining of leucocytes. The granules of the eosinophiles stained with neutral red are of conspicuous orange color, those stained with Wright's stain showed a variety of shades from light blue to purple, those stained with congo red vary from pink to red.

2. Addition of dye solution in physiologic saline (first method) or of physiologic saline alone to blood which developed Charcot-Leyden crystals in unstained specimens prevents their formation.

3. Supravital staining of eosinophiles by means of a thin dry film of dye spread over the slide (second method) does not abolish the development of Charcot-Leyden crystals. As compared with unstained preparations, neutral red has a markedly stimulating effect on their formation.

4. Regardless of the staining material used, Charcot-Leyden crystals are of the same refractile grayish to greenish shade in unstained as well as in stained preparations. The shade resembles that of unstained erythrocytes. (Fig. 1.)

5. Color, size and number of granules appear to be unchanged after formation of the crystals.

The observation that Charcot-Leyden crystals originate in individual eosinophiles leads to the obvious question why they do not develop in every eosinophile; why, as a matter of fact, a high absolute eosinophile count should be necessary for their formation. It seems permissible, therefore, to conclude that material contributed by the eosinophile represents only one, the endogenous factor necessary for the formation of the crystals.

The study of stained preparations makes it likely that granular material does not participate in the formation of Charcot-Leyden crystals. Efforts are being made to develop a reliable method of staining nuclear material, cytoplasm, and cellular material of eosinophiles, subsequent to crystal formation, as an attempt of further analysis.

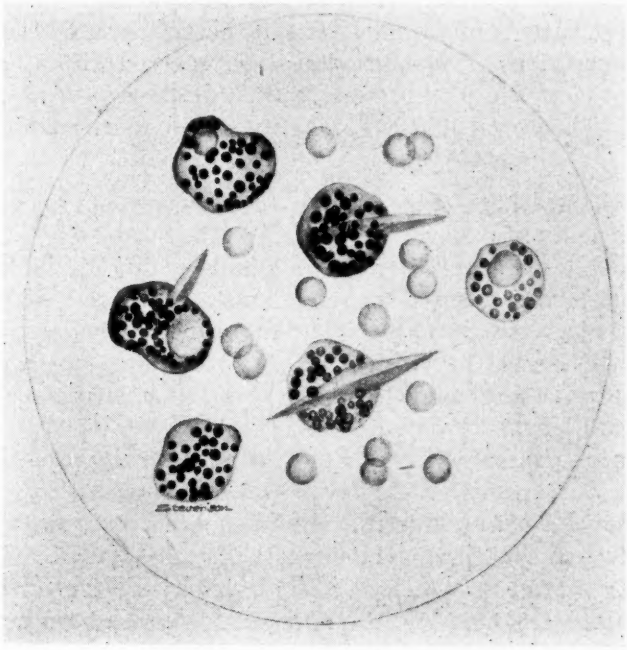

Fig. 1.—Composite drawing. Formation of Charcot-Leyden crystals in unstained and supravital stained eosinophiles. Failure to develop in the eosinophiles stained by addition of diluted dye.

II. STUDIES ON CONCENTRATIONS OF EOSINOPHILES

A. *Study on Unaltered Concentrations.*—

Experimental problem: The observation the Charcot-Leyden crystals develop in blood specimens of a high absolute eosinophile count suggested the study of their formation in concentrations of eosinophiles.

Experimental procedure: Three sources were available for the examination of eosinophile concentrations: patients with skin diseases accompanied by eosinophilia and blister formation; the cellular content of blisters produced by the application of emplastrum cantharidis N.F. 7 to the normal skin of patients with high absolute blood eosinophilia; nasal secretion of patients with abundant nasal eosinophilia. The first group included the following: patients with pemphigus, 2; with bullous dermatitis, 1. The second group included patients with Loeffler's syndrome, 2; parasites, 4; allergic disorders, 4. The third group consisted of 12 patients with allergic rhinitis exclusively. The total number of preparations was 112.

The absolute eosinophile count of the material obtained from blister fluid ranged from 18,000 to 114,000 eosinophiles per cubic millimeter. While the

blister fluid obtained from patients with pemphigus and bullous dermatitis contained practically no cells other than eosinophiles, that derived from cantharidin blisters—sixteen to twenty-four hours after application of a 2 cm. square of the plaster—contained in addition neutrophiles, monocytes, histiocytes and lymphocytes in various proportions. Only estimates could be made of the number of eosinophiles present in nasal secretions.

The concentrations of eosinophiles thus obtained were observed either in unstained preparations or on supravital slides covered with a thin dry film of neutral red: the second modification of staining described in a previous part of this paper. The period of observation was, again, fourteen days.

Results:

1. Charcot-Leyden crystals did not form in any of the preparations of blister fluid or nasal secretion.

2. Eosinophiles, separated from the liquid portion of the blister fluid by centrifuging at moderate speed for ten minutes, formed a considerable number of Charcot-Leyden crystals in material obtained from bullous dermatitis and in three out of 10 cases in which fluid of cantharidin blisters was examined.

3. Resuspension of eosinophiles in the original medium abolished their ability to form Charcot-Leyden crystals.

B. *Studies on Concentrations of Eosinophiles After Addition of Blood Serum, Blood Plasma, Erythrocytes, and Their Breakdown Products.—*

Experimental problem: Since the initial experiments reported in this paper had demonstrated that eosinophiles suspended in blood are able to form Charcot-Leyden crystals, it seemed of interest to investigate whether the addition of various fractions of blood and of its breakdown products would either prevent the development of Charcot-Leyden crystals in C-L positive centrifuged concentrations or stimulate their formation in C-L negative specimens.

Experimental procedure: One drop of the material—unaltered blister fluid, centrifuged concentrations of eosinophiles, nasal secretion—was thoroughly mixed with one drop of the blood constituent. The following fractions were used:

Human serum obtained from C-L negative blood.
Human serum obtained from C-L positive blood.
Human plasma obtained from C-L negative blood.
Human plasma obtained from C-L positive blood.
Red blood cells obtained from C-L negative blood.
Red blood cells obtained from C-L positive blood.

The mixtures were then covered, sealed, and examined in the same manner as the unaltered concentrations. The total number of specimens was 294.

Results:

1. Addition of human serum and plasma regardless of source to C-L positive concentrations of eosinophiles prevented development of Charcot-Leyden crystals; addition to C-L negative concentrations did not result in their formation (54 slides).

2. Addition of red blood cells from C-L positive blood to C-L positive concentrations of eosinophiles increased both number and size of Charcot-Leyden crystals formed; addition of red blood cells from C-L negative blood to C-L positive concentrations of eosinophiles caused either no change or an occasional reduction of the amount of Charcot-Leyden crystals formed; they did not, however, abolish their formation (38 slides).

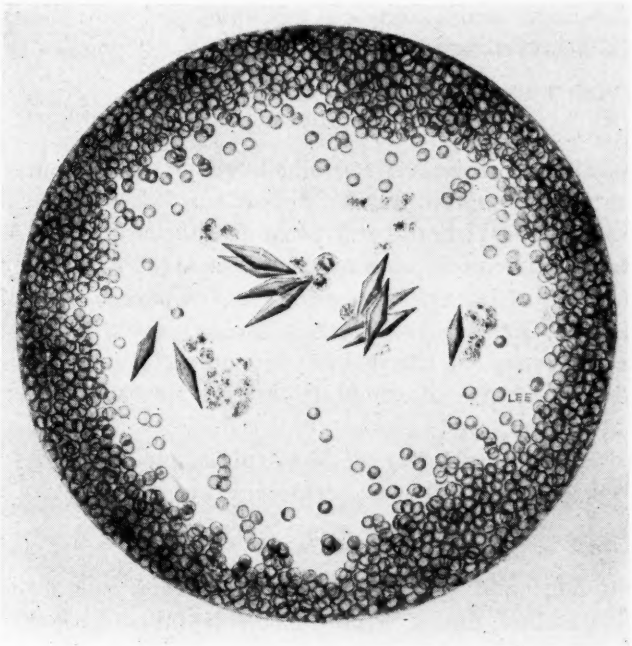

Fig. 2.—Formation of Charcot-Leyden crystals in "lakelike" areas of nasal secretion surrounded by red blood cells.

3. Addition of red blood cells from C-L positive blood to C-L negative noncentrifuged concentrations of eosinophiles obtained from blister fluid, resulted in the formation of Charcot-Leyden crystals in 7 out of 27 preparations. In all of these, the crystals developed in "lakelike" sites of the specimen where lacunae filled with eosinophiles were surrounded by a large number of red blood cells (Fig. 2). Addition of red blood cells from C-L negative blood to C-L negative noncentrifuged concentrations of eosinophiles obtained from blister fluid did not result in the formation of Charcot-Leyden crystals (18 slides). These findings are just below the conventional level of significance.

4. Addition of red blood cells from C-L positive blood to nasal secretion resulted in the formation of Charcot-Leyden crystals in 4 out of 89 preparations. In all of these, again, formation took place in liquefied areas bordered by red blood cells. Addition of red blood cells from C-L negative blood to nasal secretion did not result in the formation of Charcot-Leyden crystals (68 slides).

Further investigation of the action of breakdown products of red blood cells on concentrations of eosinophiles included the following substances:

Stroma of red blood cells obtained from C-L positive blood.
Stroma of red blood cells obtained from C-L negative blood.
Laked serum obtained from C-L positive blood.
Laked serum obtained from C-L negative blood.
Solutions in normal saline of concentrations equal to those found in red blood cells of hemoglobin and equivalent concentrations of methemoglobin, hematin, and bile pigments.

The experimental procedure remained unchanged.

Results:

1. The addition of stroma of red blood cells of either source did not prevent the formation of Charcot-Leyden crystals in C-L positive concentrations of eosinophiles (8 slides); it did not cause the formation of Charcot-Leyden crystals in C-L negative, noncentrifuged specimens (12 slides).

2. The addition of laked serum regardless of source as well as the addition of solutions of hemoglobin, methemoglobin, hematin and bile pigments prevented the formation of Charcot-Leyden crystals in C-L positive concentrations (18 slides); it did not stimulate their formation in C-L negative, noncentrifuged specimens.

3. Spectroscopic examination of blood pigments obtained from C-L positive and C-L negative blood showed identical absorption spectra.

DISCUSSION

We, on the one hand, have shown that red blood cells isolated from C-L positive blood stimulate, under certain experimental conditions, the formation of Charcot-Leyden crystals in concentrations of eosinophiles; on the other hand red blood cells from C-L negative blood as well as the stroma of these red blood cells yield negative results. The differences, however, do not assume statistical significance.

As shown on individual eosinophiles, e.g., after addition of dye solutions as well as on concentrations of eosinophiles, dilution has an inhibiting influence on the formation of Charcot-Leyden crystals.

It must be assumed that two factors are necessary for the formation of Charcot-Leyden crystals: an endogenous factor consisting of cellular material undergoing autolysis, and a contributing, exogenous factor. The action of red blood cells on concentrates of eosinophiles is only one example for this duality; the abundance of Charcot-Leyden crystals in eosinophilic conditions of the gastrointestinal tract is another.

While the mechanism proper of crystallization awaits further clarification, our findings permit us to reconcile a large number of seemingly contradictory, sometimes almost mystical facts stated in the literature on Charcot-Leyden crystals. Askanazy's[13] interesting question, for instance, why Charcot-Leyden crystals are never found in the fetal liver, in spite of the extraordinary number of eosinophiles which it contains, might be answered either by the absence

of autolytic processes within the eosinophile or by the exogenous condition required for crystallization. It also explains the outstanding observation of White,[25] who was the first American to describe the occurrence of Charcot-Leyden crystals in a leucemic patient. He found crystals in blood removed from heart, large vessels, spleen, and (though fewer) portal circulation, and adds: "In a large exudation, or abscess, situated in the cellular tissue beneath the left axilla, none were observed, although in other respects its microscopic characteristics closely resembled those of the blood."

The practical importance of our observations is negligible; they represent, however, the attempt to analyze a postmortem phenomenon further study of which will contribute to the understanding of structure, and possibly of function of the eosinophile.

SUMMARY

1. Charcot-Leyden crystals found in the blood of patients with high absolute eosinophilia, originate in individual eosinophiles. Only a minority of the eosinophiles present develops Charcot-Leyden crystals.

2. Supravital staining of eosinophilic granules does not interfere with the development of Charcot-Leyden crystals which are of the same shape and color as in unstained preparations. Dilution prevents the formation of Charcot-Leyden crystals.

3. Concentrations of eosinophiles of various sources: blister fluid, nasal secretion, failed to produce Charcot-Leyden crystals. After separation of the cellular from the liquid fraction of blister fluid, a considerable number of Charcot-Leyden crystals were seen to develop in cellular material.

4. Addition of intact red blood cells obtained from C-L positive patients with high absolute eosinophilia increased both number and size of Charcot-Leyden crystals in C-L positive, centrifuged cellular material; it stimulated the formation of Charcot-Leyden crystals in several though few instances of previously C-L negative concentrations. Addition of intact red blood cells from C-L negative patients or of the stroma of red blood cells from either source did not substantially interfere with the development in Charcot-Leyden crystals in C-L positive material, but no stimulation of development of Charcot-Leyden crystals in previously C-L negative preparations was seen.

5. Addition of substances other than red blood cells, including serum, plasma, and solutions of breakdown products of red blood cells prevented the formation of Charcot-Leyden crystals in C-L positive material and had no influence on C-L negative preparations.

6. Significance and limitations of these findings are discussed.

We are grateful for the cooperation of the Illustration Department, College of Medicine, University of Illinois, in the preparation of the figures; we also wish to acknowledge the assistance of Dr. A. G. Cole of the Department of Biological Chemistry, College of Medicine, University of Illinois, in the spectroscopic problems. Dr. W. V. Eisenberg of the U. S. Food and Drug Administration, Washington, D. C., was kind enough to give us advice on the interpretation of crystallographic behavior of crystals.

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THE VOLUMETRIC INCIDENCE OF ATMOSPHERIC ALLERGENS*†

V. Spot Testing in the Evaluation of Species

Oren C. Durham, Chicago, Ill.

THE purpose of this paper is to call attention to a little-used method of air sampling and to demonstrate its value in supplementing our information on the dispersal of air-borne allergens. The term "spot test" as here used refers to a determination of the absolute frequency of atmospheric allergens, that is, the number of particles in a given volume of air at a particular moment in a designated location. By the simple techniques here described, sampling may be accomplished in less than five minutes. The method, therefore, yields information which cannot be obtained by the gravity slide method, which affords only an approximate *mean* of the aerial incidence of various particles over a relatively long period—usually twenty-four hours—and does not give any idea of the maximal or minimal concentrations during that period.

In studying atmospheric pollen frequency, it is highly desirable to learn as much as possible about major fluctuations in the air content throughout the day. This is particularly true because the allergic response to inhaled pollen may take place within a few seconds and persist for an hour or more without further exposure.

Spot testing may be used advantageously in studying the effects of certain weather factors on the dispersal of air-borne allergens as well as in assessing the air pollution of a given indoor or outdoor location where gravity testing is not practicable. It should prove helpful in discovering sources of strictly local plant offenders. In some areas the amount of any kind of pollen suspended in the upper air is so small that the results of gravity tests have no statistical meaning. Yet, in spite of the lack of general air contamination in such areas, there may be sufficient local contact with certain active pollens to cause severe and persistent allergic symptoms in a significant number of persons. Under such conditions intensive air research requires a far more flexible technique than that afforded by the gravity method.

HISTORICAL

Short time exposures, as already noted, are not feasible with gravity slides. A few 24-hour studies have been made by using one-hour[1] or two-hour[2] slide exposures, but such tests are possible only where pollen concentrations are very high. In Blackley's[3] early atmospheric experiments he obtained air samples by means of an aspirator impinger which gave satisfactory volumetric readings in a period of an hour or less. As a result of tests made with this instrument he concluded, "In the middle portion of the day the amount of

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†From the Biological Laboratories of Abbott Laboratories.

deposit obtained in a short period was always, or nearly always, largely in excess of the average for a longer period. Then the deposit obtained in twelve hours of the day was generally much in excess of that obtained during the twelve hours of the night. Occasionally, however, the night deposit would be large, whilst that of the following day would be small in amount. As I have said above, these facts show that the amount of pollen in the atmosphere was constantly changing." However, because of the difficulty involved in operating the device, it was abandoned in favor of the 24-hour gravity method.

Duke[4] experimented with an electric fan impinger which enabled him to take five-minute air samples in any location where electric current was available. In a paper describing his technique (nonvolumetric) he referred to it as a "rapid and more accurate method of determining the pollen content of the air." He stressed the usefulness of the device, and attempted to standardize it by means of parallel gravity tests. But he neglected to carry out systematic research on any of the many problems for which the device was so obviously suitable.

In upper air sampling by aeroplane, very short exposures of oiled slides or agar plates are usually necessary because of the rapid vertical and lateral changes in the position of the plane, and because of the high rate of impingement caused by the speed of the plane. However, the pollen and fungus spore data which have been obtained from aeroplanes have seldom, if ever, been accurately volumetric, and have not as yet been coordinated with data obtained from stationary sampling devices.

The Owens' dust counter[5] or the Wells' centrifuge[6] could be used for spot testing, but no one has so far reported any aero-allergen research carried out by means of these or similar instruments. One probable reason why these devices have not been systematically exploited in this type of research is that until recently there was no reliable basis of volumetric comparison between spot reading and gravity slide figures. But now that a satisfactory gravity-volumetric technique has been devised,[7] spot readings immediately assume an important place in pollen aerobiology.

APPARATUS AND TECHNIQUE

In assessing the extreme productive ability of wind-pollinated and even insect-pollinated plants and fungi, I have used two simple devices. One of these, the Hill dust pump, consists of an air pump which impinges all captured particles in three small spots on an oiled slide. The air intake of the pump is approximately 4.5 cubic inches per stroke. By regulating the number of strokes in accordance with the degree of air pollution at a given time and place, satisfactory volumetric samples can be obtained. On a few rare occasions a single stroke of the piston has been sufficient to test the content of visible clouds of pollen, while in other situations several hundred strokes were needed. Hand-operated impinging dust counters of several designs are available, but those with attached microscopes are not very well adapted to aero-allergen research.

Another convenient device is an "air whip"—a 36-inch aluminum rod for swinging a slide in a circle with the oiled face forward. By means of repeated parallel tests, this device has been carefully calibrated against the Hill dust impinger just described, as well as against the power-driven slit impinger devised by Hawes and associates,[8] which has been used extensively in my own previously reported volumetric studies.[7, 9, 10, 11] No great difficulty has been encountered in standardizing the number of turns and the rate of rotation of the device nor the number of trips to be counted across the slide to give the approximate equivalent of the pollen content of a cubic yard of air. For ragweed pollen and those of greater size I usually make 200 turns at the rate of 100 per minute. In counting, it is only necessary to cover ten trips across the slide and to multiply the result by four. This technique allows any amount of variation according to the density of the air content. For very light fungus spores, the pick-up with this device is not as accurate as with the pump. With either device the tests can be made in a very short time. Under some conditions the dust pump is more convenient, under others the whip is preferable. Neither instrument is bulky or heavy, and counting of the samples with either is not difficult.

In securing the spot records shown in the table, every effort was made to take samples of particular species under ideal natural conditions of maximum pollen production and dispersal, numerous successive tests being made on the same occasion or on different days during anthesis. Usually the samples were taken early in the morning at the time of maturation of pollen, and, when possible, during a heavy wind on the leeward side of producing plants. Unfavorable weather conditions or other exigencies prevented adequate testing of certain species. Some common important wind-pollinated plants have not yet been tested because they do not occur or do not reach maximum development in the Chicago area where all this work has been done.

DISCUSSION OF DATA

The astonishing record for giant ragweed was obtained on Aug. 21, 1945, near Highland Park, Illinois, in the middle of a country road that runs directly through a pure stand of some 30 acres of giant ragweed. The tests were made at about 9 o'clock in the morning during the first few minutes of a sudden line squall which was preceded by ideal ripening conditions without wind. In those few minutes the windstorm dislodged the total pollen output for the day. A great yellow cloud 50 feet high was easily visible at a distance of 200 yards. The degree of air contamination on the highway at the moment of sampling may be best appreciated by the observation that while making the tests I was inhaling more than 6,000 pollen granules with every breath, or more than 100,000 granules a minute—more than a whole season's intake for the average city apartment resident. But in spite of this and other similar dense clouds of giant ragweed pollen in the country on that day, the gravity slide at the Weather Bureau station in Chicago recorded a 24-hour average of only 60 ragweed pollen grains per cubic yard of air.

TABLE I. RECORDS OF MAXIMUM AIR CONTAMINATION BY PARTICULAR SPECIES

	ROUTINE GRAVITY TESTS*		SPOT TESTS BY AUTHOR	
	NO. PER CU. YD. OF AIR	PLACE	NO. PER CU. YD. OF AIR	REMARKS
Ragweeds				
Short (*Ambrosia elatior*)	5628	Indianapolis	78,000	In wheat stubble. Inadequate record.
Giant (*Ambrosia trifida*)			9,600,000	In a visible cloud of pollen 20 feet from weeds
Cocklebur (*Xanthium spp.*)	8	Kansas City, Mo.	440	Test made in very weedy cornfield
Burweed Marsh Elder (*Iva xanthifolia*)	556	Scottsbluff, Nebr.	160,000	Test made adjacent to small patch
Composites				
Goldenrod (*Solidago canadensis*)	5		192	Three feet above weeds
Sunflower (*Helianthus annuus*)	–		12	On leeward side of large acreage
Wild Lettuce (*Lactuca canadensis*)	–		0	In large patch
Fleabane (*Erigeron canadensis*)	–		0	In large patch
Chamomile (*Anthemis cotula*)	–		0	In large patch
Chicory (*Cichorium intybus*)	2		184	In heavily grown vacant lot
Coneflower (*Lepachys columnifera*)	–		0	In patch of many acres
Daisy Fleabane (*Erigeron ramosus*)	–		60	Test on windy day in large field
Ox-eye Daisy (*Chrysanthemum leucanthemum*)	–		504	Three feet above blossoms in high wind
Coreopsis (*Coreopsis lanceolata*)	–		0	Leeward side of large patch
Dandelion (*Taraxacum officinale*)	–		2	Ten-acre lawn. Heavy crop. Windy.
Dog Fennel (*Eupatorium capillifolium*)	107	New Orleans	–	
Unidentified composite	177	Oklahoma City	–	
Grasses				
Orchard Grass (*Dactylis glomerata*)	–		2,000,000	In a visible cloud of pollen
Canada Bluegrass (*Poa compressa*)	–		460,800	Four feet above heavy growth
Redtop (*Agrostis palustris*)	–		328,000	Leeward side of meadow
Timothy (*Phleum pratense*)	–		66,000	In a large field. Unsatisfactory record.
Bluegrass (*Poa pratensis*)	–		14,000	In a large field. Unsatisfactory record.
Millet (*Setaria italica*)	–		9,840	In a large field
Corn (*Zea mays*)	–		4,640	Roadside, leeward side of field.

*References are noted and credit is thus extended for gravity records which are quoted or interpolated from published reports other than those of the author. All other gravity figures are from the author's own records made over a period of more than two decades. These studies have been made possible by assistance in air sampling by the observers of the United States Weather Bureau, the Canadian Meteorological Service, and numerous cooperating allergists and interested laymen in all parts of the United States and Canada.

TABLE I. RECORDS OF MAXIMUM AIR CONTAMINATION BY PARTICULAR SPECIES (CONT'D)

	ROUTINE GRAVITY TESTS*		SPOT TESTS BY AUTHOR	
	NO. PER CU. YD. OF AIR	PLACE	NO. PER CU. YD. OF AIR	REMARKS
Oats (*Avena sativa*)	–		1,360	Three feet above blossoming heads
Sudan Grass (*Holcus sorghum sudanensis*)	–		704	Leeward side of heavy growth
Western Wheat Grass (*Agropyron smithii*)	–		520	Leeward side of small patch
Wheat (*Triticum sativa*)	–		80	Three feet above heads
Unidentified mixed grasses	250	Kansas City, Mo.	–	
Unidentified mixed grasses	770	Minneapolis[12]	–	
Chenopodiales				
Mexican Firebush (*Kochia scoparia*)	165	Scottsbluff, Nebr.	86,400	Three feet above weeds
Russian Thistle (*Salsola pestifer*)	110	Aberdeen, S. Dak.	–	
Western Water Hemp (*Acnida tamariscina*)	528	Omaha	1,800	Leeward side of heavy growth
Water Hemp (*Acnida tuberculata*)	–		12,000	Three feet above weeds
Lamb's Quarters (*Chenopodium album*)	15	Indianapolis	768	In large weed patch
Pigweed (*Amaranthus retroflexus*)	15	Cleveland	800	In large weed patch
Miscellaneous Weeds				
Hemp (*Cannabis sativa*)	116	Omaha	–	
Hop (*Humulus japonica*)	134	Philadelphia[13]	–	
Nettle (*Urtica gracilis*)	56	Omaha	46,665	Leeward side of small patch
	184	Minneapolis[12]		
English Plantain (*Plantago lanceolata*)	30	Philadelphia[13]	3,840	Four feet above heavy growth
	136	Minneapolis[12]		
Red Sorrel (*Rumex acetosella*)	25	Yonkers[14]	50	Unsatisfactory spot record
	574	Minneapolis[12]		
Trees				
Pine (*Pinus spp.*)	387	Grand Lake, Colo.	12,000	Under Austrian pine tree
Alder (*Alnus spp.*)	200	Richmond, Va.[15]	–	
Elm (*Ulmus americana*)	618	Worcester, Mass.[16]	15,000	Under tree, windy
	1,755	Minneapolis[12]		
Elm (*Ulmus crassifolia*)	176	Dallas	–	
Cottonwood (*Populus virginiana*)	302	Dallas	930	Under trees. Unsatisfactory
	3,287	Minneapolis[12]		
Box Elder (*Acer negundo*)	909	Minneapolis[12]	1,168	Under tree
Sugar Maple (*Acer saccharum*)	–		300	Under tree
Soft Maple (*Acer saccharinum*	–		256	Under tree
Ash (*Fraxinus americana*)	370	Minneapolis[12]	12,000	Under tree
Sycamore (*Platanus occidentalis*)	597	Philadelphia[13]	–	
Oak (*Quercus spp.*)	650	Yonkers[14]	1,000	Unsatisfactory record
	2,809	Minneapolis[12]		
Walnut (*Juglans nigra*)	51	Charlotte, N. C.	–	
Hickory (*Hicoria spp.*)	125	Kansas City, Mo.	–	
Birch (*Betula papyrifera*)	238	Worcester, Mass.[16]	1,400	Under tree
Pear (*Pyrus communis*)	–		948	Under tree
Willow (*Salix nigra*)	–		198	Under tree

TABLE I. RECORDS OF MAXIMUM AIR CONTAMINATION BY PARTICULAR SPECIES (CONT'D)

	ROUTINE GRAVITY TESTS*		SPOT TESTS BY AUTHOR	
	NO. PER CU. YD. OF AIR	PLACE	NO. PER CU. YD. OF AIR	REMARKS
Wild Plum (*Prunus americana*)	–		32	Leeward side of bushes
Paper Mulberry (*Broussonetia papyrifera*)	335	Charlottesville, Va.[17]	–	
Miscellaneous Entomophilous Plants				
Sweet Clover (*Melilotus alba*)	–		992	Leeward side of heavy growth
Red Clover (*Trifolium pratense*)	–		0	In large field
Black Mustard (*Brassica nigra*)	–		96	Two feet above the weeds
Opium Poppy (*Papaver somniferum*)	–		27	Two feet above the blossoms
Knotweed (*Polygonum spp.*)	–		0	Above heavy growth
Asparagus (*Asparagus officinalis*)	–		0	Above heavy growth
Tamarisk (*Tamarix gallica*)	–		0	Beside shrubs
Wild Rose (*Rosa blanda*)	–		0	Six inches from flowers
Lilac (*Syringa vulgaris*)	–		0	Beside shrubs
Fungi				
Alternaria spp.	14,600	Moorhead, Minn.	1,360	In a cornfield. Inadequate record
			675,000	In dust from combine (harvester-thresher)
Hormodendrum spp.	20,000	Minneapolis	869,000	In dust from combine (harvester-thresher)
Helminthosporium spp.	30	Moorhead, Minn.	177,327	In dust from combine (harvester-thresher)
Stem Rust (*Puccinia graminis*)	6,800	Goodland, Kan.	–	
	10,380	Moorhead, Minn.		
Corn Smut (*Ustilago zeae*)	500,000	Wayzata, Minn.[18]	–	

No such ideal opportunity has subsequently presented itself for testing short ragweed. There was no visible cloud of pollen over the stubble of the old wheat field where I got my best short ragweed sample. The cocklebur spot figure is probably a fair one. By comparing it with records for any of the other ragweeds and with those of insect-pollinated composites one may safely conclude that under ordinary conditions the possibility of specific exposure to cocklebur pollen is almost as remote as of exposure to pollen of goldenrod.

In considering the orchard grass record cognizance must be taken of the fact that the sample was secured under conditions similar to that which produced the high figure for giant ragweed. Visible clouds of pollen could be seen blowing across the street from a vacant lot that supported an almost pure growth of orchard grass. No visible clouds of pollen were encountered while testing any of the other heavy producing grasses. It was extremely difficult to obtain high spot counts of corn pollen even on windy days either outside a cornfield or between the corn rows. The western wheat grass record is probably typical of the performance of most of the wild grasses.

Water hemp spot records much higher than mine could probably be obtained in the cornfields and creek bottoms of Iowa and eastern Nebraska. Under

favorable conditions the western species (*Acnida tamariscina*) actually rivals the productive performance of the ragweeds. I doubt whether much better pigweed or lamb's quarters records can be obtained. The English plantain figure is also as high as could reasonably be expected.

Results with the tree tests were as a whole quite disappointing, as weather conditions were often very unfavorable. For some species higher counts have actually been obtained with 24-hour gravity slides, particularly at Minneapolis, where Dr. Rosendahl's sampling station must have been located in a grove of oak or cottonwood trees.

The testing of miscellaneous insect-pollinated plants other than composites has proved what we already suspected—that most such plants are negligible sources of air contamination. The case for rose pollen as an air contaminant is very poor indeed.

My only worth-while spot records for the fungi were obtained in the heavy clouds of dust produced by a combine while in operation in an oat field. These findings should be supplemented by tests in elevators and feed mills as well as in undisturbed grain fields. My highest Alternaria spot record made under natural conditions in Illinois was 13,600, which is only slightly less than my highest 24-hour average as found by gravity testing in the center of Moorhead, Minnesota.

SUMMARY

By using two simple sampling devices I have tested the allergen producing ability of more than 60 plant species and several fungi, including many of the more common hay fever offenders and a number of related insect-pollinated species.

As a result of repeated spot testing of the air in the immediate vicinity of single plants, small plots or extensive acreages, some very striking figures for maximum production have been obtained. These are tabulated and in almost every case compared with maximum gravity data (stated on volumetric basis) as previously reported for these plants in areas where they are most abundant.

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DISCUSSION

DR. MATTHEW WALZER (Brooklyn).—As an allergist and as a member of the Pollen Survey Committee of this society, I am indebted, in many ways, to Mr. Durham. It is indeed an unusual state of affairs to have so many who are interested in the treatment of hay fever depend on the contributions of a single man for so much of the information which makes such treatment possible.

This is the fifth in a series of studies by Durham which, undoubtedly, will be historic in this field. It affords a very quick way to obtain, in a few minutes, information about what is happening at a particular spot at a particular time under particular conditions. If you tried to get that information from the gravity slide, it would, of course, be impossible for reasons which have already been pointed out.

These spot-testing procedures have wide applications and advantages. They can be used indoors as well as outdoors. They give us considerable information about certain agents which some think are very important aero-allergens; but, when tested with these instruments, prove to be insignificant.

Of course, procedures of this kind would not be very practical without standard techniques with which they may be compared. That emphasizes the importance of the attempts of the Pollen Survey Committee to standardize counting techniques so that we may all talk the same language and understand each other. It is probably superfluous to point out that these methods for obtaining spot information are not intended to replace the standard pollen counting techniques which Mr. Durham as Technical Chairman of the Pollen Survey Committee is doing so much to promote.

Original Articles

SENSITIZATION EXPERIMENTS IN GUINEA PIGS FOLLOWING INJECTION WITH SIMIAN OR RODENT POLIOMYELITIS VIRUS*†

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VIRAL diseases are rarely accompanied by a state of hypersensitivity which is demonstrable by the usual local or systemic allergic tests. Notable exceptions are those induced by the viruses of vaccinia or lymphogranuloma in which such reactivity can be elicited routinely in the course of human or experimental infection. The limited occurrence of anaphylactic phenomena in this field is unexplained, particularly if one considers that most viral diseases are followed by an abundant output of antibodies and that the infectious process carries with it a widespread and intense cellular reaction. That some viruses are intrinsically capable of causing in a sensitized test animal, like the guinea pig, a typical anaphylactic response upon reintroduction of the same virus substrate was shown for bacteriophage[1] and tobacco mosaic virus.[2] On the other hand, no evidence of general or local hypersensitiveness could be detected in guinea pigs following active or passive sensitization with vaccinia virus.[3]

In the case of poliomyelitis, previous attempts to demonstrate specific hypersensitiveness in infected monkeys[4] or in human convalescents[5, 6] by various methods have yielded inconclusive results. However, the problem seemed worthy of reinvestigation in an experimental animal, such as the guinea pig, which is well suited for anaphylactic study and, at the same time, is susceptible to systemic infection with certain strains of rodent poliomyelitis virus.[7, 8, 9] The fact that such infection, whether symptomatic or asymptomatic, is regularly followed by the development of a high virucidal antibody titer in the serum of the injected guinea pigs (1,000 paralytic virus doses neutralized by intracerebral test and 1,000,000,000 paralytic virus doses neutralized by intraperitoneal test in mice[10]) provided additional incentive for undertaking this work. A series of experiments were therefore, carried out in which guinea pigs were sensitized by injection with live virus of simian, murine, or cavian origin. After a proper interval, the sensitized animals were reinjected with the corresponding live viruses in homologous as well as in heterologous combinations. Guinea pigs which received normal monkey or mouse tissue for the sensitizing or shocking injections were included for control purposes. A brief description of the technical details follows.

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TABLE I. RESULTS OF REINJECTION OF SIMIAN OF RODENT VIRUS IN SENSITIZED GUINEA PIGS

SENSITIZATION	TOTAL NUMBER OF GUINEA PIGS	REINJECTION WITH SIMIAN VIRUS (I. V.)				REINJECTION WITH NORMAL MONKEY CORD (I. V.)				REINJECTION WITH MOUSE VIRUS (I. V.)				REINJECTION WITH NORMAL MOUSE BRAIN (I. V.)				REINJECTION WITH CAVIAN VIRUS (I. V.)			
		NO.	POS.	DOUBTFUL	NEG.	NO.	POS.	DOUBTFUL	NEG.	NO.	POS.	DOUBTFUL	NEG.	NO.	POS.	DOUBTFUL	NEG.	NO.	POS.	DOUBTFUL	NEG.
Simian virus (i.c./i.p.)	34	6	4	0	2	4	3	0	1	17	0	5	12	4	0	0	4	3	0	0	3
Murine virus (i.p.)	42	18	1	2	15	3	0	0	3	9	7	0	2	4	3	0	1	8	0	0	8
Cavian virus (i.p.)	30	5	0	0	5	2	0	0	2	15	0	3	12	4	0	0	4	4	0	1	3
Normal monkey cord (i.c./i.p.)	14	2	1	0	1	3	1	0	2	6	0	0	6	3	0	0	3	–			
Normal mouse brain (i.c./i.p.)	8	2	0	0	2	2	0	0	2	2	2	0	0	2	2	0	0	–			
—	19	3	0	0	3	3	0	0	3	7	0	0	7	3	0	0	3	3	0	0	3

Positive: Typical bronchospasm, convulsions and anaphylactic death within three to five minutes.
Doubtful: Slight coughing and sneezing, occasional jerky movements, two to three degree drops of temperature, no anaphylactic death.
Negative: One to two degree drop of temperature, no symptoms, no anaphylactic death.

EXPERIMENTAL WORK

Young guinea pigs of mixed stock, weighing between 175 and 200 Gm., were employed for this work. Simian virus (Aycock strain) was used in form of a centrifuged 10 per cent viral monkey cord suspension and murine or cavian virus (MM strain)[8] in form of centrifuged 10 per cent brain suspensions, harvested either from paralyzed mice or guinea pigs; normal tissue suspensions were prepared in a similar manner. Sensitization with simian virus or with normal tissues was carried out by intracerebral injection (0.1 c.c.) in conjunction with two intraperitoneal injections (1 c.c.), one dose given simultaneously, the other twenty-four hours later. In the case of rodent virus, two intraperitoneal injections only were administered in order to minimize the heavy paralytic losses resulting from intracerebral virus injection. No untoward symptoms were seen following injection of the normal tissues, and none of the guinea pigs injected with simian virus showed any unusual manifestations. However, in a combined group of 150 guinea pigs injected peripherally with either murine or cavian virus, 78 (52 per cent) developed paralysis while 72 (48 per cent) remained symptomless. The high mortality rate of the paralyzed guinea pigs (about 90 per cent) considerably reduced the number of animals receiving rodent virus which were available for reinjection. Further losses in all groups occurred from occasional intercurrent infections. After a free interval of 3 to 4 weeks all sensitized surviving guinea pigs were reinjected intravenously (1 c.c.) with one of the several viral or normal preparations. The animals were carefully observed for immediate symptoms of shock, and the different grades of response were recorded. Guinea pigs which had received for the first time live rodent virus during the shocking injection were further observed for the ultimate development of paralysis. The results are given in Table I.

It will be seen from Table I that animals sensitized to simian virus could be severely shocked, in a high proportion of the cases, upon reinjection with simian virus; however shock was observed with about the same frequency upon reinjection of normal monkey cord. Similarly, severe anaphylactic symptoms were readily produced in animals sensitized to murine virus following reinjection with murine virus, but shock occurred at least as often after rejection of normal mouse brain. On the other hand, animals sensitized to cavian virus failed, with two exceptions, to respond to reinjection of either simian or rodent virus. In keeping with this experience, cross experiments in which animals sensitized to simian virus were reinjected with murine virus, and, vice versa, gave essentially negative results. It appears, finally, that guinea pigs sensitized to normal tissues of monkey or mouse could be shocked not only with homologous normal tissues, but also with corresponding viral tissues of the two animal species. Control tests, in which the various preparations were injected intravenously into normal guinea pigs, proved the absence of primary toxicity.

DISCUSSION

The observed facts leave no doubt that, whenever hypersensitivity occurred with any degree of regularity, it was produced, not by the virus, but by the anaphylactogenic action of the respective animal tissues. The isolated instances in which shock occurred under conditions precluding the participation of tissue antigenicity are too few in number and too indistinct in character to detract from this statement. Under the conditions of these experiments, therefore, virus did not sensitize to itself in a manner discernible by classical anaphylactic shock, even though the guinea pigs infected with rodent virus were known to carry a high concentration of virucidal antibodies in their serum.

One explanation for this failure may be that, despite its high biological potency, chemically defined virus in infected unpurified brain is but an infinitesimal part of the total brain proteins.[11] It is doubtful whether the use of chemically purified virus as sensitizing agent would have produced a more intense response than what was obtainable in these experiments as the result of systemic infection, with the accumulation of large amounts of active virus in neural and nonneural tissues. A possibility that focal reactions—of the tuberculin type—may have occurred in the central nervous system cannot be ruled out. However, histologic examination of the cords of three guinea pigs, sensitized to and reinjected with murine virus, revealed no fresh inflammatory lesions. Against such cellular hypersensitivity also speaks the fact that guinea pigs which had been sensitized with simian virus and reinjected intravenously with murine virus developed paralysis (12 paralyzed of 15 injected) in about the same percentage as did normal guinea pigs of comparable age (five paralyzed of seven injected) following primary intravenous injection of murine virus.

It remains to point out the apparent ease with which typical shock could be elicited in guinea pigs sensitized to normal tissues upon reinjection with viral tissues of the same animal species. This observation would suggest either that nervous tissues, as the result of viral infection, had undergone only minimal biochemical changes, insufficient to entail the development of a new immunological specificity in the substrate, or, as previously mentioned, that virus protein was quantitatively only an insignificant component of the total brain proteins undetectable even by the anaphylactic test.

SUMMARY

Previous contact with simian or rodent poliomyelitis virus fails to induce in the guinea pig a state of hypersensitivity which can be demonstrated by the classical anaphylactic test upon reintroduction of homologous or heterologous virus.

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SPIROMETRIC EVALUATION OF BENADRYL IN ASTHMA*

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THE antihistamine properties of benadryl (beta-dimethylaminoethyl benzhydryl ether hydrochloride [A-524]) were first investigated by Loew, Kaiser, and Moore.[1, 2] Some of the earliest clinical investigations of benadryl were reported by Curtis and Owens[3] in cases of acute and chronic urticaria. Since then, there have been numerous reports[4-9] of its efficacy in hay fever, vasomotor rhinitis, asthma, migraine, urticaria, Ménière's disease, and allied disorders. There has been no consistency in the reports concerning the effectiveness of benadryl in asthma and few objective observations[10] have been published. The purpose of the present study is to evaluate the effects of benadryl on individual attacks of asthma by employing spirometric methods to derive objective data.

PROCEDURE

Sixteen cases of extrinsic bronchial asthma were studied. These patients had received treatment at the Charity Hospital of Louisiana at regular intervals for periods varying from two to ten years prior to this study. Most of them had been ward patients on numerous occasions, and all had been attending the Allergy Clinic. Pulmonary function studies were performed shortly after the onset of a moderately acute attack in each case. A modification of the Benedict-Roth respirometer[11] was employed. Tracings were made prior to, at thirty minutes, and one hour after taking 100 mg. of benadryl orally. From these tracings measurements were made of the pulmonary volumina and its subdivisions, respiratory rate, minute ventilation, oxygen consumption, and the ventilatory equivalent (liters of air ventilated for each 100 c.c. of oxygen absorbed). The distance between the expiratory level of the vital capacity breath and the expiratory level of the preceding normal breath was determined from the tracings. This value, expressed in milliliters of air, was called the expiratory differential. The degree of emphysema was estimated, as suggested by Cournand and coworkers[11] from the retardation and prolongation of expiration, inability to return to the initial expiratory level on successive deep breaths, and alterations in the maximum breathing capacity.

The dosage of benadryl selected, 100 mg., was considered to be the largest single amount which could be given routinely without encountering a high incidence of unpleasant reactions.

In five of these sixteen cases, the same studies were performed following the administration of 0.3 mg. of epinephrine hydrochloride subcutaneously and 0.25 Gm. of aminophylline intravenously.

In addition to the above objective determinations, each patient was observed and questioned concerning subjective alleviation of dyspnea. The development

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TABLE I. SPIROMETRIC RESULTS FOLLOWING BENADRYL

PATIENT	MINUTE VENTILATION*			VITAL CAPACITY*			TIDAL AIR*			RESPIRATORY RATE			EXPIRATORY DIFFERENTIAL*		
	0	½ HR.	1 HR.	0	½ HR.	1 HR.	0	½ HR.	1 HR.	0	½ HR.	1 HR.	0	½ HR.	1 HR.
1	6.76	6.88	6.98	2.71	2.50	3.01	0.49	0.49	0.43	14	14	16	0.45	0.30	0.68
2	8.95	9.03	9.87	1.92	2.22	2.29	0.36	0.38	0.38	25	24	26	0.45	0.34	0.58
3	11.96	10.15	9.07	0.96	1.07	1.28	0.43	0.40	0.30	28	25	30	0	0	0
4	8.03	8.97	9.44	1.28	1.20	1.50	0.30	0.32	0.33	27	28	29	0.28	0.20	0.43
5	5.48	5.51	6.74	1.07	1.22	1.58	0.41	0.46	0.35	14	12	19	0.47	0.24	0.38
6	6.34	2.05	5.28	1.69	1.17	1.56	0.45	0.34	0.39	14	12	14	0	0	0
7	18.84	8.35	11.08	2.50	2.60	3.50	0.87	0.67	0.89	22	13	13	0.68	0	0.32
8	4.02	3.82	3.87	1.03	0.81	0.66	0.26	0.23	0.21	16	17	19	0	0	0
9	6.85	7.08	7.69	1.54	1.17	1.30	0.40	0.36	0.39	17	20	20	0.66	0.24	0.28
10	11.34	Status asthmaticus		1.37	Status asthmaticus		0.38	Status asthmaticus		30	Status asthmaticus		0.49	Status asthmaticus	
11	7.38	6.58	5.70	0.79	0.81	0.79	0.35	0.30	0.28	21	22	20	0.11	0.24	0.15
12	6.16	6.92	5.13	0.75	1.03	1.09	0.33	0.38	0.26	19	18	20	0.15	0.11	0.02
13	18.86	13.13	13.07	1.50	1.54	1.86	0.50	0.44	0.38	38	30	34	0.17	0.11	0.34
14	13.58	13.46	9.51	3.78	3.31	3.23	0.77	0.75	0.70	17	19	14	0.90	0.55	0.45
15	8.28	6.52	9.42	3.31	3.23	2.80	0.53	0.43	0.45	15	15	20	1.13	0.81	0.26
16	7.99	8.98	8.54	1.35	1.05	1.15	0.36	0.45	0.43	22	20	20	0.15	0.15	0.15

*Expressed in liters.

of any significant symptoms during the course of the experiment was recorded. These studies were conducted in the Lung Station, where environmental conditions were kept constant.

RESULTS

The observed effects of benadryl are presented in Table I. It will be noted from this table that there was no consistent effect on any of the measurements. In the individual cases, a considerable variation existed between the thirty-minute and one-hour measurements. One case developed status asthmaticus thirty minutes following administration of benadryl and required hospitalization. In the other fifteen cases the vital capacity was decreased in six and increased in nine. The maximum increase in vital capacity was 45 per cent of the initial measurement preceding benadryl in one case; however, only four of the nine patients had an increase above 25 per cent of their initial vital capacity. The tidal air was diminished in nine cases and increased in six. The maximal increase in volume of the tidal air was 24 per cent of the original value in one case. Only two cases showed an increase above 12 per cent. The respiratory rate was increased in five cases and decreased in ten cases. Of these ten cases, one showed a decrease of nine respirations per minute. In the other eight cases only one decreased more than four respirations per minute. The minute ventilation was increased in eight cases and decreased in seven. The increase amounted to 25 per cent in one case. None of the other seven cases increased above 17 per cent. The tracings of eleven patients failed to show an increase in the expiratory differential, whereas five had an increase at the end of one hour. Five cases showed spirometric evidences of moderate to marked emphysema. One of these five cases showed a definite reduction in the spirometric evidences of emphysema following benadryl.

Only one of the sixteen cases showed a response to benadryl characterized by a combination of increase in vital capacity, increase in tidal air, increase in the minute ventilation, increase in the expiratory differential, and no increase in the respiratory rate. In addition, four other patients showed spirographic evidence of varying degrees of improvement.

The oxygen consumption determinations were omitted from consideration for two reasons: (1) Spirometric tracings show a two-thirds greater variation than gas analysis;[12] (2) some of our patients were slightly cyanotic and probably had some degree of anoxemia, which resulted in a steeper initial slope of the oxygen absorption curve. This variability in the measurement of oxygen consumption rendered the measurements of the ventilatory equivalent unsatisfactory.

Six of the sixteen patients stated that there was complete relief of dyspnea. Observations of these patients revealed a considerable decrease in the signs of dyspnea, although some respiratory difficulty persisted.

Various side effects were observed and are listed in Table II.

In contrast to the above findings are the results of the administration of epinephrine and aminophylline in five of these same cases, as shown in Table III. The vital capacity, tidal air, minute ventilation, and expiratory differential

TABLE II. SIDE REACTIONS TO BENADRYL

REACTION	NUMBER OF CASES
Vertigo	5
Dry mouth	8
Drowsiness	7
Anxiety	2
Weakness	3
Epigastric pain	1
Nausea	2
Difficulty in coordination	1
Feeling of mild inebriation	12
Blurred vision	1
Tinnitus	1
Extreme dyspnea	4
Palpitation	2
Precipitation of status asthmaticus	1

were increased in all five patients. The respiratory rate was decreased in four and remained the same in one. The maximum increase in vital capacity was 100 per cent, the minimum increase was 24 per cent. The tidal air increased more than 64 per cent in three cases; the remaining two showed increases of 19 per cent and 37 per cent respectively. The minute ventilation varied from a maximum increase of 66 per cent to a minimum increment of 3 per cent. All five cases showed a uniform response to epinephrine and aminophylline characterized by a combination of increase in vital capacity, increase in tidal air, increase in minute ventilation, increase in expiratory differential, and no increase in the respiratory rate. Although Mudd[13] did not consider the expiratory differential, the remainder of the findings are in complete agreement with his results.

TABLE III. SPIROMETRIC RESULTS FOLLOWING EPINEPHRINE AND AMINOPHYLLINE

PATIENT	MINUTE VENTILATION*		VITAL CAPACITY*		TIDAL AIR*		RESPIRATORY RATE		EXPIRATORY DIFFERENTIAL*	
	A†	P‡	A	P	A	P	A	P	A	P
3	10.00	16.70	0.66	1.26	0.38	0.64	26	26	0.13	0.30
6	6.86	7.05	1.20	1.84	0.34	0.47	20	15	0.04	0.13
8	5.64	6.32	0.85	1.71	0.23	0.40	24	16	0.17	0.21
10	25.16	26.08	2.71	3.35	0.66	0.79	38	33	0.85	0.92
16	7.99	11.96	1.35	1.82	0.36	0.60	22	20	0.15	0.41

*Expressed in liters.
†a = Before epinephrine and aminophylline.
‡p = Fifteen minutes after epinephrine and aminophylline.

Only one of these five cases showed spirographic evidence of emphysema, which was not markedly affected by the administration of epinephrine and aminophylline.

DISCUSSION

From our studies we are unable to find any spirometric evidence of a consistent effect of 100 mg. of benadryl given orally in individual, moderately acute attacks of asthma. No attempt was made to evaluate the prolonged use of benadryl. When adrenalin and aminophylline were given, a consistent increase in the vital capacity, tidal air, minute ventilation, expiratory differential, and no increase in the respiratory rate occurred.

After the administration of an effective bronchodilator, the acute attack of asthma probably subsides in two phases with a variable period of transition between phases. The first phase is concerned with the initial alleviation of bronchiolar spasm. During this period labored breathing tends to persist, especially in those patients suffering from anoxemia. Spirography at this time will show an increase in the tidal air, minute ventilation, vital capacity, and often the oxygen consumption. The expiratory differential will either increase or remain unchanged. The second phase is concerned with the perpetuation of relief of bronchiolar spasm and the return of respiratory effort to a resting level. Spirography at this time may show a reduction in oxygen consumption, tidal air, respiratory rate, and minute ventilation. The vital capacity should remain increased over the initial level, and the expiratory differential should be increased if the lung has not lost its elasticity.

Since the second phase is of variable onset and duration, evaluation is best performed during phase one. Obviously, sufficient time must be given for absorption and action of the drug being investigated. The time of onset of phases one and two and the duration of the transitional period will depend upon the effectiveness and speed of action of the drug, in addition to the patient factors concerned.

The increase in tidal air and vital capacity following administration of epinephrine and aminophylline is largely due to relief of bronchiolar spasm. During the attack of asthma there is a temporary acute emphysema with an increase in the residual air at the expense of the vital capacity, especially the complemental fraction. When bronchiolar spasm is relieved, the obstruction is removed, and normal exchange of air occurs with an increase in the vital capacity and a reduction in the residual air.[14] This relief of bronchiolar obstruction decreases the anoxemia and CO_2 retention, when present, by reducing the residual air and establishing normal pressure gradient relationships between the gases in the alveoli and the pulmonary capillaries. The return to normal of these biochemical changes is reflected by a decreased respiratory rate. Undoubtedly, psychic factors also exert a considerable influence in decreasing the respiratory rate.

The minute ventilation will depend upon the relationship between the tidal air and the number of respirations per minute. When epinephrine and aminophylline are given, the tidal air is increased and the number of respirations decreased in phase one. The minute ventilation will be augmented if the increase in tidal air more than compensates for the reduction in respiratory rate. The increased value for the expiratory differential may be explained by a relief of the temporary overdistention of the alveoli, thus allowing the normal elasticity of the lung to function.

All of the above effects were variable following benadryl administration. The mechanism of action of benadryl has been postulated to involve a displacement of histamine or a competition[15] with histamine for its site of action. The release of histamine or a histamine-like substance at the bronchioles has been held by some to be responsible for the initiation of bronchiolar spasm. In our

experiment the effect of benadryl, in the dosage used, regardless of the mode of action, was not sufficient consistently to overcome the bronchiolar spasm encountered.

These experiments were performed shortly after the onset of the attack, and the factor of bronchiolar obstruction due to mucus plugging was at a minimum.

Many of our patients stated that they obtained considerable benefit from benadryl, and six of these cases who were moderately dyspneic at the beginning of the experiment had a marked subsidence of dyspnea following the administration of benadryl, despite the presence of only slight changes in their spirometric tracings. These patients had been observed on numerous occasions before and had never shown relief of dyspnea within periods of one or two hours unless some specific medication was given.

There is a voluminous literature relative to psychic factors in asthma. Whereas we do not believe that such factors play a primary etiologic rôle, we do feel that they may exert considerable influence both in the precipitation and perpetuation of some attacks and in the treatment of some asthmatics. Perhaps the fact that these patients expected relief from benadryl was of some importance in those who derived subjective relief and moderation of dyspnea. This is suggested by the fact that subjective improvement with benadryl in six patients was not accompanied by objective evidence of improvement in three, whereas there was complete correlation between subjective and objective relief with the use of epinephrine and aminophylline.

As suggested by Cournand and associates,[11] and again more recently by Herxheimer,[16] spirometric methods afford a simple, reliable means of quantitating the effects of various drugs in asthma. We feel that the subjective evaluation of any drug given to asthmatic patients is of little scientific value. Since epinephrine and aminophylline afford both subjective relief and objective spirometric evidence of improvement in most asthmatics, we feel that these drugs should be used as a base line by which newly introduced drugs, for which bronchodilator effects are claimed, may be quantitatively evaluated.

SUMMARY

1. Spirometric studies were performed on sixteen patients, thirty minutes and one hour following the administration of 100 mg. of benadryl orally.

2. When compared with the initial spirometric tracings, no consistent changes were noted in the vital capacity, tidal air, minute ventilation, expiratory differential, respiratory rate, or degree of emphysema following benadryl.

3. Following the administration of epinephrine and aminophylline to five of these sixteen patients, there was a uniform increase in the vital capacity, tidal air, minute ventilation, expiratory differential, and no increase in the respiratory rate.

4. Six of the patients derived subjective benefit with decrease in dyspnea following the use of benadryl. In three of these, spirometric data were directly opposed to the subjective report. In one case the same response was obtained as found after the administration of epinephrine and aminophylline.

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SENSITIZATION TO PENICILLIN

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MANY new drugs bring with them a train of cutaneous reactions. Numerous reports have appeared as to penicillin which at once raise the question: Does treatment of superficial lesions with penicillin sensitize some individuals sufficiently to prevent internal treatment which might later be critically needed for some general infection?

On this account, special attention was paid to cutaneous reactions during a study of the treatment of superficial infections with penicillin.[1] Patients who had received penicillin previously were tested for sensitivity before treatment; those who appeared irritated by penicillin during treatment were tested immediately, and all returning for checkup two weeks after stopping treatment were given patch "site" and intradermal tests. Those who reacted were also tested, if possible, by intramuscular injection.

REACTIONS TO TESTS

Patch tests were made by bandaging a small amount of ointment under cellophane to the inner side of the upper arm or ventral surface of the forearm for forty-eight hours. The earlier tests were made with carbowax base containing 500 units to 10,000 units per Gm.; later tests with an emulsion containing 100,000 units of penicillin in 0.4 c.c. of water and 0.6 Gm. of lanolin.

Sensitized patients developed local areas of dermatitis which were usually vesicular and usually faint. In the few cases in which two concentrations were compared, no reactions were produced with the stronger ointment unless some degree of reaction could be seen under the weaker. The 100,000 unit preparation appeared the most satisfactory for routine testing on account of the more definite response it elicited.

"Site" tests were made by applying penicillin ointment for twelve to twenty-four hours to an area previously treated by penicillin. Reactions consisted in a dermatitis.

Intradermal tests were made with 0.1 c.c. of physiologic saline containing 10,000 units of penicillin per cubic centimeter. A few immediate reactions were encountered but the following data, unless specified, refer to reactions observed after twenty-four hours.

The various types of reaction to these intradermal tests have been described by Welch and Rostenberg.[2] Sensitized individuals showed erythematous patches, at least 5 mm. in diameter and usually distinctly infiltrated as

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†With the technical assistance of Gerbert C. Rebell and R. Bruce Ledin.

in tuberculin reactions. Many, however, showed a finely vesicular or crusted surface such as results from a patch test. Some of these reactions formed plaques of severe dermatitis 3 to 4 cm. in diameter. From these and from some smaller reactions, wavy red lines of lymphangitis extended up the arm. The morphology of these reactions as well as their close correlation with patch tests showed that many indicated epidermal sensitization. The simple papular reactions probably indicated the tuberculin type of hypersensitivity, although this distinction could not be drawn in all instances.

An unusual feature was the occasional delay of response. In two cases, where the test appeared quite negative in twenty-four hours, there was a distinct reaction in four and five days, respectively.

Three other types of reaction to intradermal tests were observed which probably did not indicate sensitization. One was a minute spot of intense erythema or purpura 2 or 3 mm. in diameter which appeared in twenty-four hours and often left a dot of pigmentation; another a tense vesicle of similar size which occasionally resulted in a minute shallow ulceration. Neither type was associated with other evidence of sensitization, and they were believed to be irritations. These reactions occurred in groups, a number being seen in one week after which several weeks would pass before more were observed. This suggested that they were due to something in certain lots or vials of penicillin, but they could not be traced to any particular lot number. They occurred with three different commercial brands, but never in tests with crystalline penicillin G.

A third type of reaction was a wheal 10 or 15 mm. in diameter often surrounded by erythema, which appeared twenty to thirty minutes after an intradermal injection. In unselected hospital patients tested intradermally, 45 per cent of 55 who had previously been treated with penicillin reacted in this manner, and 35 per cent of 43 who had never received penicillin also reacted. On this account they could not be considered evidence of sensitization.

More intense swellings occasionally occurred within ten minutes of injection, which probably indicated endothelial sensitization, but as they never formed sharply bordered wheals with pseudopods, it seemed impossible to distinguish them from the more frequent nonallergic immediate reactions.

Passive transfer was attempted with the serum of four patients, all of whom had given a delayed reaction, and one an unusually pronounced immediate reaction to intradermal tests. Their sera were injected into the forearms of two recipients and the sites tested twenty-four hours later. The results were completely negative.

TABLE I. CORRELATION OF INTRADERMAL TESTS WITH CRYSTALLINE PENICILLIN G AND COMMERCIAL PENICILLIN

Crystalline	+	Commercial	+	13 cases
Crystalline	+	Commercial	–	1 case
Crystalline	–	Commercial	+	6 cases
Crystalline	–	Commercial	–	57 cases
		Total		77 cases

Tests With Crystalline Penicillin G.—Of 19 patients who gave positive intradermal reactions to commercial penicillin, reactions to an equal number of units (1,000) of crystalline penicillin G were positive in 13. This indicated that the majority of the reactions obtained to commercial preparations were due to penicillin itself and not to any impurity in the preparation.

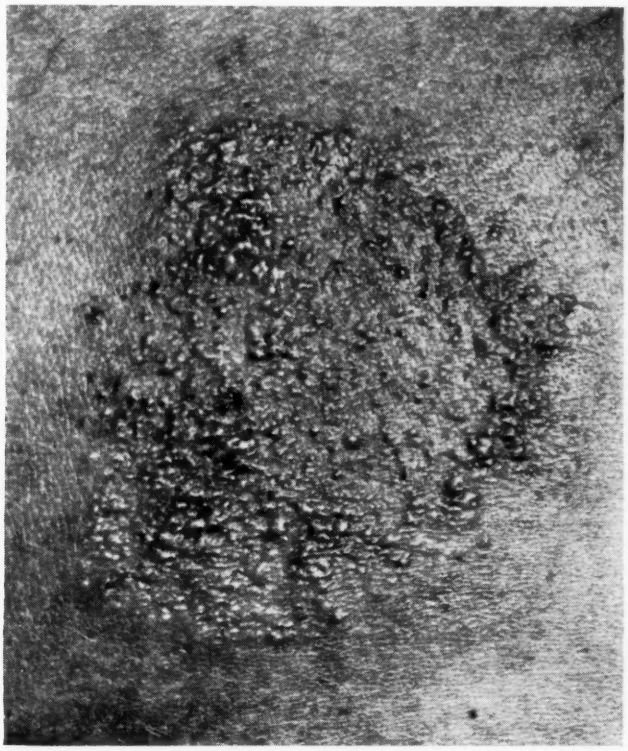

Fig. 1.—Reaction to patch test with penicillin ointment, 100,000 units per c.c. in a patient with penicillin dermatitis.

The explanation of the six disagreements is not clear. The reaction to commercial penicillin in these patients may have been due to an impurity or may conceivably have been due to penicillin F or X. It is more probable that the explanation lies in the difference in reaction obtained to the two types of preparations. Solutions of crystalline penicillin never caused the small macules or vesicles which were frequently seen after intradermal tests with commercial penicillin and which were recorded as nonspecific irritation. The reactions to the pure substance which were regarded as positive were, as a rule, less pronounced than those to the commercial preparation in the same patient. Possibly this was because they were not reinforced by nonspecific irritants present in the latter solutions.

Intramuscular tests were made by injecting 100,000 units of penicillin in saline and observing any generalized or local reactions in twenty-four hours. In cases suspected of being highly sensitive, a smaller dose was administered.

TABLE II. CORRELATION OF INTRAMUSCULAR TESTS

PATCH TEST	INTRADERMAL TEST	NUMBER OF CASES	INTRAMUSCULAR TESTS	
			POSITIVE	NEGATIVE
+	+	22	17	5
+	−	1	0	1
−	+	10	2	8
−	−	57	4	53
		90	23	67

Of 90 patients tested, 23 gave what was considered a positive response. In five cases the reaction was an urticaria, in three generalized patches of erythema, in three intense generalized pruritus, all of which probably indicated endothelial sensitization. Four others showed localized vesicular erythemas of the type described later, and ten showed focal reactions consisting of an acute dermatitis in areas previously treated with penicillin, which indicated local epidermal sensitization. In eight this focal reaction was the only response and there was no reaction in untreated areas of skin.

Correlation of Tests.—The result of the patch and intradermal tests done simultaneously was usually the same. Of 405 cases, 50 were positive and 335 negative to both tests. Fourteen gave a positive reaction to intradermal, but negative to patch test. Six were positive to patch but negative to intradermal. This correlation as well as the frequent eczematous character of responses to intradermal tests indicated that either method might be used to detect epidermal sensitization. The intradermal tests were easier to perform and to read.

REACTIONS TO PENICILLIN THERAPY

Previous observers have described urticaria after systemic penicillin therapy, dermatitis after local therapy, and also erythematous and vesicular eruptions. In this study 618 patients were treated with penicillin, 70 by oral and/or intramuscular routes, 492 locally, and 56 by both local and systemic methods. Sixty-five (11 per cent) showed one of the following reactions: generalized urticaria, 1 case; disseminated erythematous or vesicular eruptions, 8 cases; and localized dermatitis, 56 cases.

Generalized Urticarial or Pruritic Reactions.—Lyons[3] reported the occurrence of urticaria in about 5 per cent of cases treated parenterally with penicillin. In our series urticaria appeared in one patient under treatment, in three during attempted desensitization, and in seven after tests. Two had previously received only local treatment, which indicates that it is possible to sensitize the endothelium to penicillin by applications to the skin surface. The urticarial reactions were induced in five by intramuscular, in two by intradermal injection, and in one by oral administration, but in none by surface application. Only one case was severe enough to demand hospitalization. Two eruptions were accompanied by mild asthma. Most subsided within twenty-four hours, but in one case wheals continued to appear for four days. The urticarial response was seldom immediate, and in one case appeared four days after the responsible intradermal injection.

Three additional patients complained of severe generalized pruritus after intramuscular injections. This was considered equivalent to an urticaria, as another patient with this symptom developed frank urticaria after a subsequent injection.

Fig. 2.—Erythemato-vesicular eruption on the palm, following intramuscular injection of penicillin in a sensitized patient.

Typical immediate wheal reactions to intradermal tests were not seen in these patients. Failure to detect such reactions may have been due to the frequent occurrence of the indefinite wheals described above, which were apparently not expressions of specific hypersensitivity. Several patients gave

delayed reactions to intradermal or patch tests, but these were doubtless related to epidermal sensitization and not to their urticaria.

DISSEMINATED ERYTHEMATOUS OR VESICULAR ERUPTIONS

Various extensive cutaneous reactions which were difficult to classify occurred after treatment or tests.

Four instances probably resulted from endothelial sensitization. These patients showed on the trunk large areas of erythema somewhat edematous or papular, one after intramuscular treatment, one during oral, and two after application of ointment to a distant lesion. The two former gave no reaction to patch tests, and one failed to react when 100,000 unit solution was painted on the site of the previous erythematous reaction. This indicated that their sensitization was not epidermal. The two latter gave positive patch tests and reacted violently to local treatment, which was evidence of epidermal sensitization in addition to probable sensitization of their vascular endothelium.

Four other patients developed erythematous and vesicular eruptions on the face, fingers, or groin, which may have been due to local epidermal sensitization. Two occurred after intramuscular injection, and two after surface treatment of a distant area. All of these patients had positive patch and intradermal tests which showed their epidermis was sensitized. Similar reactions have been described by Graves,[4] Lamb,[5] and Cormia,[6] who have emphasized their resemblance to dermatophtids.

After intramuscular tests two other patients developed isolated wheals, one on the sides of the fingers and one on the groin, which on account of their localization seemed related to the vesicular eruptions just described.

Localized Dermatitis.—These reactions consisted in the exacerbation of a pre-existing dermatitis or development of a dermatitis in an area under local treatment. They varied greatly in severity from a slight exacerbation of the lesion, sometimes so slight that it was difficult to identify it as a reaction, to a severe dermatitis with edema. Only one reaction in this group was severe enough to necessitate hospitalization. In addition to the reactions resulting from treatment, four patients who were engaged in filling capsules with penicillin developed a blepharitis or mild dermatitis of the hands similar to the reactions described by Pyle.[7]

Reactions of this type were much more frequent in patients with a preexistent eczematous dermatitis, as can be seen in Table III. There was no evident relation to atopy. Only two patients gave a history of asthma, one of hay fever, and one of infantile eczema.

Several patients who were quite intolerant of surface applications of penicillin were treated intramuscularly or orally with benefit. In 13 of 23 patients, intramuscular tests caused no reappearance of the dermatitis, although some continued to react to local applications.

Irritations.—Six of the patients who developed dermatitis from application of penicillin to their lesions gave no reaction to patch and intradermal tests. These were believed to be nonallergic irritations. Five had been treated with

TABLE III. FREQUENCY OF CLINICAL REACTIONS TO PENICILLIN THERAPY*

DIAGNOSIS PRIMARY INFECTIONS	NUMBER OF CASES	PER CENT REACTING
Impetigo Contagiosa	52	8
Impetigo Bullosa	35	0
Ecthyma	33	6
Folliculitis of Beard	50	14
Folliculitis Elsewhere	28	7
Furunculosis	21	0
Hidrosadenitis	9	0
SECONDARY INFECTIONS IN:		
Dermatophytosis of Feet	226	4
Intertrigo	15	20
Eczematous Dermatitis	144	25
Acne	23	9

*This includes urticarial and erythematous, as well as eczematous reactions.

10,000-unit ointment, the sixth with a 100,000 unit solution. Two of them later tolerated applications of 500-unit ointment.

It may be that some of these apparent irritations were actually sensitizations. Seven additional cases were at first suspected of being examples of irritation, because patch and intradermal tests made at the time of their clinical reaction were negative. A positive intradermal or patch test was obtained, however, on a later occasion, and they have consequently been tabulated as instances of sensitization. It is probable that had tests been repeated in all six cases classed as irritation, some would have given a positive response showing that they were specifically sensitized to penicillin. Others may have failed to react to tests because their sensitization was limited to certain areas of skin.

Six other patients who reacted in a similar manner during treatment disappeared before tests were made, so that it could not be determined whether they were irritations or true sensitizations.

Herxheimer-like Reactions.—Three patients with folliculitis of the beard showed intense local reactions with redness, marked edema and increase in pustulation. Two occurred on the first and one on the fifth day of treatment. Two gave positive patch and intradermal tests, but the third was repeatedly negative. All were later treated without reaction and with improvement or complete healing of their infection. It seemed probable that these were Herxheimer phenomena rather than sensitizations.

Gluteal Reactions.—Another type of reaction should be noted which may not be due to penicillin. Two patients on about the fourteenth day of intramuscular injections of penicillin in beeswax and oil developed huge indurated and exquisitely tender lesions in the buttocks. The overlying skin was dusky, and the lesions resembled Arthus phenomena or the necroses occasionally seen from bismuth injections. They, however, subsided in two or three days under hot compresses without breaking down of tissue. These two accidents occurred on the same day, and no similar reaction was seen at any other time. It is suspected that they were due to some fault in melting the penicillin preparation.

In summary, it may be said that reactions to penicillin therapy, while sufficient to arrest treatment, were seldom enough to cause great discomfort. They subsided usually in three or four days, though in ambulant patients they were occasionally difficult to treat. The important fact, however, is that there were no instances of severe generalized completely disabling dermatitis, such as is seen after treatment with arsenic, gold, the sulfonamides, atabrine, and other drugs. Their bearing on the possibility of treating the same patient with penicillin at some later period will be discussed later.

Localized Sensitivity.—It was evident in these cases that epidermal and endothelial sensitization did not always occur together. The former, at least, often involved only restricted areas of the epidermis. The clearest evidence as to this were the focal reactions which occurred after intramuscular injections. Only the previously treated areas of skin responded.

In a few cases, tolerance to treatment differed in different areas, especially in one patient who developed a dermatitis from treatment of the groin with penicillin ointment, although the same treatment was well tolerated in the axillas. In some cases, inequality in reaction was probably due to difference in permeability of the skin, as in the case of the patient who responded positively to a patch test on the arm, though he tolerated treatment well on the sole of his foot. In other instances, however, the reaction seemed clearly due to local sensitivity. This probably explains the occasional reaction to treatment of patients who gave negative patch or intradermal tests.

Loss of Sensitization.—It was noted by Lyons[3] that patients who developed urticaria from parenteral injection, could often be treated shortly afterward without reaction. It was evident in this series that epidermal sensitization to penicillin was also frequently transient, especially in patients showing mild reactions. Seven patients who reacted at one time to penicillin could later be treated without reaction. Some tolerated the strongest ointment.

The results of skin tests also demonstrated loss of sensitivity. Of twelve patients given "site" tests, a week or more after their clinical reaction, ten reacted, but two were negative. Two other patients who had given positive patch tests, later gave negative tests four and six weeks later. Twenty-seven patients whose intradermal tests were positive were retested two to twelve weeks after termination of treatment. Eight (30 per cent) then gave no response.

Many patients showed variations from week to week in response to skin tests, which at first appeared quite irregular, but may have represented waves of transient sensitization. One patient whose tests are shown in Table IV had an eczematous dermatitis of the hands and feet, secondary to dermatophytosis. He had previously been treated with penicillin ointment. In the first week of resumed treatment with 500-unit ointment, the dermatitis on his fingers became exaggerated. A second trial of local treatment in the third and fourth week again excited his dermatitis. In the fifth week, an intramuscular injection produced an intense flare-up in the treated areas, but the following week a similar injection produced only transient redness and itching of the feet and fingers, which subsided in fifteen minutes. A few days later 500-unit ointment

was applied without reaction. Treatment was continued eventually with 10,000-unit ointment and the signs of infection disappeared. The underlying eczema cleared up under treatment with coal tar. Two and three months later the patient was retested by patch, by intradermal, and intramuscular injection, and by application of penicillin to the site of his previous lesions and showed no reaction.

TABLE IV. VARIATIONS OF REACTION TO PENICILLIN IN A CASE OF ECZEMATOUS DERMATITIS

REACTIONS TO	WEEK OF OBSERVATION									
	1ST	2ND	3RD	4TH	5TH	6TH	7TH	8TH	15TH	17TH
Local Application	+		+	+		−	−	−	−	−
Intramuscular		−			+	±	−		−	−
Intradermal Test	−	−	−	+	+−	−+−	−	±	−	−
Patch Test		−	−−	−	−−	−	±	±	−	−

Desensitization.—In seven severely sensitized patients, intramuscular desensitization was attempted on approximately the following schedule:

Two days 10 units intramuscularly 3 times a day
Two days 100 units intramuscularly 3 times a day
Two days 1,000 units intramuscularly 3 times a day
Two days 10,000 units intramuscularly 3 times a day
Two days 20,000 units intramuscularly 3 times a day
Two days 40,000 units intramuscularly 3 times a day

Two of the patients broke out with urticaria on reaching the 1,000-unit doses, and a third on reaching 10,000 units, a fourth reached the 100-unit doses without reaction, but failed to continue. The remaining three patients were able at the end of the period of desensitization to take therapeutic doses without reaction. Trial of desensitization by the oral route would be desirable.

Pre-Existing Sensitization.—Eleven patients reacted at the outset of their treatment. Ointment had been applied to ten, and one had been given capsules also. The remaining case had been given an intramuscular injection. Six gave positive intradermal tests at the time of their reaction. Five were then negative, but four developed positive tests one to three weeks later. The remaining case developed a vesicular eruption on his hands after intramuscular injection which indicated local hypersensitivity. Six of these patients had been previously treated with penicillin, and three had had reactions to their previous treatment. This doubtless explains their sensitization. The remaining five were sensitized to penicillin in some undetermined manner. These primary sensitizations have been noted by others and have caused much speculation. Graves and others[4-6] have suggested the hypothesis that such patients were sensitized by a previous attack of dermatophytosis and that their reaction was to some broad antigen common to all fungi.

PROBABILITY OF SENSITIZATION FROM LOCAL TREATMENT BY PENICILLIN

The best evidence we have as to the risk of sensitizing a patient was obtained from site, patch, and intradermal tests made about fourteen days after treatment had stopped (Table V *A*, *B*, and *C*). These were made on all patients who could be recalled for follow-up observation and the material is consequently unselected as to type or severity of case.

TABLE V. REACTIONS TO SITE PATCH AND INTRADERMAL TESTS

*A. Frequency in Different Types of Infection**

DIAGNOSIS PRIMARY INFECTIONS	FOURTEEN-DAY TESTS†				TOTAL TESTS‡		
		PER CENT OF CASES REACTING				REACTING	
	CASES TESTED	SITE	PATCH	INTRA-DERMAL	CASES TESTED	PATCH	INTRA-DERMAL
Impetigo Contagiosa	27-29§	7%	7%	10%	32-33§	9%	12%
Impetigo Bullosa	15	0	0	0	15	0	0
Ecthyma	16	6%	12%	12%	18	11%	11%
Folliculitis of Beard	16	6%	12%	19%	24	13%	21%
Folliculitis Elsewhere	16	6%	6%	6%	19	5%	5%
Furunculosis	5	0	0	0	5	0	0
SECONDARY INFECTIONS IN:							
Dermatophytosis of Feet	143	6%	10%	10%	148	11%	10%
Intertrigo	10	30%	30%	30%	11	27%	27%
Eczematous Dermatitis	63-65§	22%	27%	26%	82-84§	27%	28%
Acne	3	0	0	0	4	0	0
Total	314-318§	10%	13%	14%	358-361§	13%	15%

B. According to Duration of Treatment

DURATION OF TREATMENT	FOURTEEN-DAY TESTS†				TOTAL TESTS‡		
		PER CENT OF CASES REACTING				REACTING	
	CASES TESTED	SITE	PATCH	INTRA-DERMAL	CASES TESTED	PATCH	INTRA-DERMAL
1- 7 Days	192-193§	8%	11%	10%	198-199§	12%	11%
8-14 Days	78	11%	13%	16%	78	12%	14%
15 Days and over	52-55§	15%	20%	24%	82-84§	22%	25%

C. According to Route and Strength of Treatment

TREATMENT	FOURTEEN-DAY TESTS†				TOTAL TESTS‡		
		PER CENT OF CASES REACTING				REACTING	
SYSTEMIC	CASES TESTED	SITE	PATCH	INTRA-DERMAL	CASES TESTED	PATCH	INTRA-DERMAL
Intramuscular	7	0	0	0	7-8§	12%	0
Oral	5	0	0	0	5	0	0
Intramuscular and Oral	8	0	0	0	14	0	0
Total	20	0	0	0	26-27§	4%	0
LOCAL*							
Units							
100	22	5%	5%	5%	22	5%	5%
500	138	7%	9%	9%	141	10%	9%
10,000	129-132§	12%	16%	17%	160-163§	18%	19%
100,000	25-26§	25%	27%	31%	35	20%	28%

*All cases tabulated received local treatment, a few received oral and/or intramuscular treatment also.

†All tests were made after treatment, the majority on the fourteenth day.

‡"Fourteen Day Tests" plus tests made during treatment.

§A few cases did not receive all three tests.

It is evident from these tests as it was from tabulation of the frequency of clinical reactions (Table III) that the risk of sensitization was much greater in a patient who already had an eczematous dermatitis. This evidence may be weighted by the fact that these eczematous cases were obstinate and were treated on an average for a longer period than the other infections observed.

TABLE VI. SENSITIZATION REACTIONS OBSERVED IN TREATMENT OF PYOGENIC INFECTIONS OF THE HANDS AND FEET

TREATMENT AGENT	NUMBER OF CASES	SUSPECTED REACTIONS	DEFINITE REACTIONS
Penicillin	297	0	10.0%
Sulfathiazole	353	6%	0.3%
Zephiran	149	13%	2.0%
Zinc Peroxide	139	6%	0.0%

This factor would not, however, influence the greater frequency with which clinical reactions occurred in these eczematous patients early in the course of treatment. In Table V, *B* these reactions are retabulated according to the length of the treatment given, and it is evident that the longer treatment is continued, the greater is the chance of sensitization.

In Table V, *C* they are tabulated according to the route and intensity of treatment. No sensitizations of the epidermal type were produced in patients treated by oral and/or intramuscular administration alone. This is in accordance with all reports on large series of cases treated intramuscularly, which agree that such an event is rare. The tabulation of cases treated by ointments of different strengths include patients who received oral or intramuscular therapy in addition to these surface applications. The strength of ointment indicated is the greatest used at any time, in some cases only for one or two days of a long course of treatment. The results indicate that increasing the strength of the ointment increases the probability of sensitization. It is evident that epidermal sensitivity occurs rarely except after surface application of penicillin and probable that the stronger the preparation used and the longer it is applied, the greater the probability that sensitization will develop.

The occurrence of reactions to treatment in the form of dermatitis in 9 per cent of the 618 patients treated and of positive reactions to patch tests in 13 per cent of the 318 cases tested after treatment shows that penicillin is an excellent sensitizing agent for the epidermis. Comparison of the reactions observed in this series with other similar cases treated by other agents is shown in Table VI. This summarizes the sensitizations observed in treatment of pyogenic infection in all types of dermatoses of the feet and hands, including eczematous dermatitis.

The reactions counted as "suspected" consisted of increased itching or exaggeration of a preexistent dermatitis. In view of the many reports on the frequency of sensitization to sulfathiazole, it is surprising that we encountered only one definite case in 353 patients treated topically. This one patient, as a matter of fact, had been sensitized by oral treatment which he had received previously and to which he had reacted. From our experience penicillin would seem a far more frequent sensitizer than sulfathiazole. On the other hand, it should be emphasized that when dermatitis does result from sensitization to sulfathiazole, it is likely to be far more severe.

The main consideration as to the importance of sensitization induced by local penicillin therapy is whether it would make the patient incapable of tolerating intramuscular therapy which might be necessary for a grave infec-

tion at some later period. The probability of such intolerance resulting is diminished somewhat by the fact that only 58 per cent of 33 patients whose sensitivity was demonstrated by intradermal or patch tests reacted when tested by intramuscular injection. The probability is further reduced by evidence brought out above showing that epidermal sensitization to penicillin is frequently local and frequently transitory. Moreover, the cutaneous reactions resulting from such sensitivity are rarely severe. It is probable that in most of the sensitized cases in this series, the reaction to intramuscular therapy would not have been of such severity as to prohibit treatment urgently needed. There were, however, four or five cases (about 0.8 per cent) so severely sensitized that intensive intramuscular therapy would be impossible if their sensitivity persisted.

Though slight, this possibility of seriously sensitizing the patient, must be considered in starting therapy. In impetigo, ecthyma and infected dermatophytosis without evidence of eczematization, the necessary period of treatment is usually short and the risk of sensitization seems negligible. It would seem practically nonexistent in systemic treatment of furunculosis and hidroadenitis. In the treatment of any case with eczematous eruptions, however, the risk of sensitization should be weighed against the probability of benefit. In such cases penicillin therapy should probably be limited to those showing an active infection and to those in whom other available methods have failed.

SUMMARY

1. Urticaria, erythemato-vesicular reactions or dermatitis occurred in 11 per cent of cases treated for cutaneous infections with penicillin.

2. After treatment with penicillin ointment, 13 per cent of cases were shown by patch tests to be sensitized.

3. In patients with eczematized lesions, sensitization was demonstrable in 27 per cent.

4. Sensitization was often localized to limited areas of skin and was frequently transient. No severe generalized and completely disabling eruptions were encountered.

5. Sensitization severe enough to prevent or prohibit systemic treatment with penicillin was encountered in less than 1 per cent of the cases.

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EXPERIMENTAL RESEARCH ON A NEW SERIES OF CHEMICAL SUBSTANCES WITH POWERFUL ANTIHISTAMINIC ACTIVITY: THE THIODIPHENYLAMINE DERIVATIVES*

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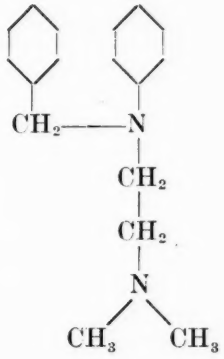
THE fields of pharmacology and therapeutics have lately been enriched with a new group of substances of highly specific activity, the synthetic antihistamines. This discovery is the outcome of the research of French authors carried out mainly during the occupation. As evidenced by the numerous articles that have appeared in France and abroad, especially in the United States, this work is of interest.

The first products studied in 1937 by Staub and Bovet,[2, 3] especially thymoxyethyldiethylamine (929 F) and diethylaminoethyl ethylaniline (1571 F) had only a mediocre antihistaminic activity: they barely protected the guinea pig against three or four lethal doses of histamine, and were highly toxic both to the animal and to man. Nevertheless, some therapeutic trials were carried out, but it was soon realized that the very poor tolerance exhibited to these products did not permit any therapeutic result, and the trials were soon abandoned.

The subject of antihistamines seemed to have reached an impasse when we took up its study in 1940.[1] In collaboration with Walthert, we have studied a series of 64 substances belonging to the aniline, pyridine, and quinoline series, synthetized in the laboratories of the Societé Rhone-Poulenc, by P. Koetchet, Mosnier, and Bouvier. Among the substances studied, the one found to be the most active in all the pharmacological tests was N′dimethyl N′aminoethyl N′benzyl aniline:



It was introduced into therapeutics under the name of "Antergan." This substance protects the guinea pig against 50 or 60 lethal doses of histamine. It neutralizes the effects of histamine on smooth muscle both in vitro and in vivo.

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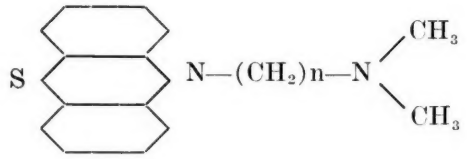
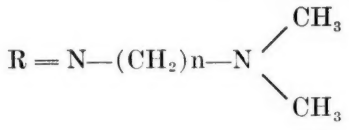
It protects the animal against the bronchospasm caused by inhalation of histamine aerosol, and counteracts the vascular effects of histamine in various animal species. However, these substances are practically without effect on the action of histamine on the various external secretions—gastric, salivary, and pancreatic.

Accordingly, 2339 RP proved to be amazingly active in the syndromes of experimental hypersensitiveness, and in a dosage of 0.5 to 1 mg. per kilogram protected the guinea pig against fatal anaphylactic shock. The same was true for the dog. These experimental results, now confirmed by numerous authors in France, the United States, Switzerland, and Belgium, are now further supported by the successful results obtained in the therapy of allergic conditions with these products. Thus, the physiologic and clinical interest aroused by these new substances has been justified. More recently, other compounds of similar action have been reported in France, the United States, and Switzerland.

Continuing our work in this field, we have recently discovered a new chemical series of substances* possessing strong antihistaminic and antianaphylactic activity: these are the derivatives of thiodiphenylamine. Their general formula may be written:



The rule which we stated for the aniline derivatives,[1] and which has been confirmed by Bovet, Horclois, and Walthert,[6] and Mayer, Huttner, and Scholz,[7] for the pyridine series, occurs again in the thiodiphenylamine compounds:



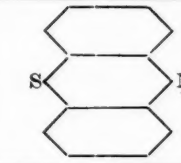
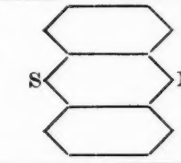
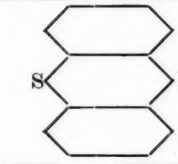
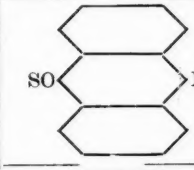
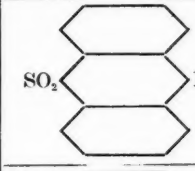
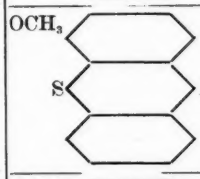
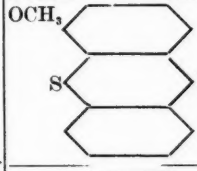
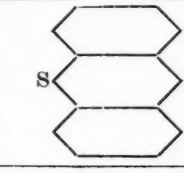
namely, that the most active products possess the chain:

We have studied the action of a certain number of the thiodiphenylamine derivatives upon the different histamine effects, and also upon the phenomena of experimental anaphylaxis. We have also studied the variations resulting from modification of the chemical structure of the different compounds of this group of substances. The following table shows the activity of these substances with respect to three fundamental tests:

1. Antihistaminic activity, as expressed by the number of lethal doses of histamine that the animal can resist after an hypodermic injection of 20 mg. per kilogram of the antihistamine.

*These compounds were synthesized by P. Charpentier in the laboratories of the Societé Rhone-Poulenc.

TABLE I. COMPARISON OF THE ACTIVITY OF THE ANTIHISTAMINES DERIVED FROM THIODIPHENYLAMINE

NAME OR NUMBER	FORMULA	ACTIVITY ON ISOLATED INTESTINE, IN PER CENT	ACTIVITY IN HISTAMINE INDUCED ASTHMA IN PER CENT	ANTIHISTAMINIC POWER (LETHAL DOSES)	
				RABBIT	GUINEA PIG
Antergan 2339 R.P.		100	100	20-25	60
3015 R.P.		100	100	175	400-500
3276 R.P.		70			20
3277 R.P.		100	200	400	1500
3283 R.P.		8-10	20		50
3289 R.P.		45	50		200
3298 R.P.			100		100
3299 R.P.		50	100-200		400
3300 R.P.		50	< 2		

2. Antibronchospasmic activity, as expressed by the minimum dose of the antihistamine which protects the guinea pig against the bronchospasm provoked by histamine aerosol.

3. Antagonistic effect on smooth muscle, as measured by the dose required to obtain an antagonistic effect against histamine on the guinea pig isolated intestine.

To these three, one may add the antianaphylactic effect, which will be discussed later.

In our tests, we have used Antergan as the standard for comparison of the activity of the various substances.

PHARMACOLOGIC ACTIONS

We wish here to summarize briefly the essential pharmacologic properties of this new series of antihistamines and to bring out their common and divergent characteristics, by comparison with the previously known antihistamines.

1. *Antihistaminic action.*—The antihistaminic action of a substance may be determined by the degree of protection against the toxic properties of histamine that is afforded by preliminary administration of the substance in question. This activity can be expressed as the number of lethal doses of histamine that can be tolerated by an animal protected by previous injection of the antihistamine. This test, which is certainly the most specific criterion, has been reported by Staub[3] in determining the effectiveness of the derivatives of 929 F and of 1571 F. We have used it for the Antergan and thiophenylamine series. In these trials, the antihistamine has been given by hypodermic injection, in a dose of 20 mg. per kilogram. Twenty minutes later the histamine dihydrochloride was given by intravenous injection. In Table II we have compared the antihistaminic activity of several previously known antihistamines with that of the thiodiphenylamine derivatives.

TABLE II. NUMBER OF LETHAL DOSES TOLERATED BY THE ANIMAL AFTER ADMINISTRATION OF THE ANTIHISTAMINE

	THYMOXYETHYL DIETHYLAMINE (929 F) (2) (3)	N'DIMETHYLAMINOETHYL N BENZYLANILINE (2339 RP ANTERGAN) (1)	N'DIMETHYLAMINOETHYL N-P-METHOXY α AMINOPYRIDINE (2786 RP NEO-ANTERGAN) (6)	DIMETHYL AMINOETHYL THIODIPHENYLAMINE (3015 RP) (4) (5)	DIMETHYL AMINO-2 PROPYL 1 THIODIPHENYLAMINE (3277 RP) (4) (5)
Guinea pig	2	50-60	80	400	1500-1600
Rabbit	–	20-25	30	100-125	400-450
Dog	–	2-3,5	2-2,5	12	15-18
Mouse	–	increases toxicity	increases toxicity	no effect	no effect

*We consider the lethal dose of histamine dihydrochloride given intravenously to be: 0.6 mg./kg. for the guinea pig, 1.5 mg./kg. for the rabbit, 10 mg./kg. for the dog.

One cannot help but be impressed by the marked antihistaminic activity with respect to histamine exhibited by the derivatives of thiodiphenylamine, especially 3015 and 3277 RP. This activity is found with all animal species sensitive to histamine. In the mouse, which is very insensitive to histamine, this protective action of the antihistamines is absent.

The mechanism of this protective action is still unexplained. The trials so far carried out confirm the opinion which we expressed in 1942,[1] namely, that the protective does not depend entirely upon the abolition of the bronchospasm caused by histamine. Using injections of adrenaline or aleudrine, considerably larger than those needed to protect the animal against the bronchoconstrictor effect of histamine, we have been unable to protect the guinea pig against even two lethal doses of histamine. We shall deal later with this topic.

2. *Antagonistic effect on the bronchi of the guinea pig.*—Applying to this animal the aerosol technique that we developed and reported in 1942[1] for the study and standardization of the antihistamines, we have been able to observe that preliminary subcutaneous injections of approximately 1 mg. per kilogram of the thiodiphenylamine derivative 3277 RP protects the animal completely against the severe asthmatoid crises otherwise provoked in this animal by two or three minutes of histamine aerosol inhalation. One new point special to this series of substances is worthy of emphasis; the action of 3277 RP lasts much longer, twice or three times as long as that of 2339 RP and 2786 RP. Thus, an injection of 1 mg. per kilogram of 3277 RP protects against histamine induced bronchospasm for about nine hours while 2339 RP or 2786 RP, even in larger doses, are not effective for more than three or four hours.

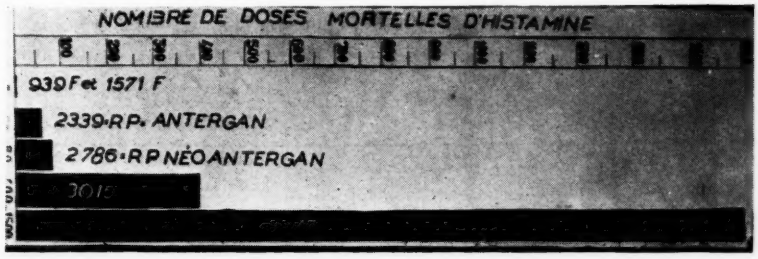


Fig. 1.—Comparison of the antitoxic activity against histamine of some antihistaminе derivatives in the guinea pig.

We have verified these observations as well as the specificity of the antihistaminic activity of this series of substances by recording in situ the tone of the guinea pig bronchi, using the technique which we have described previously.[1]

3. *Antagonistic action on excised guinea pig intestine.*—The contraction caused by histamine in the isolated gut of the guinea pig can be suppressed by the thiodiphenylamine derivatives (3015 RP and 3277 RP) in concentrations of the same order as those of the histamine employed. With this test, the new compounds do not show any greater activity than 2339 RP and 2786 RP; i.e., they act in concentrations of the order of 10^{-8}. It seems that in this organ, we have reached the ultimate dilution possible for the histamine-antihistamine reaction—a dilution probably depending upon the molecular concentration on the surface of the organ.

4. *Antagonistic action on the guinea pig uterus.*—It is known that with the guinea pig uterus it has been impossible to demonstrate the existence of an

antihistaminic effect for the reason that the derivatives of the phenolic esters as well as the Antergan series of substances possess oxytocic properties of their own. We were later able to show[8] that this antagonism does exist *in vitro* as well as *in vivo,* but to demonstrate it on the excised uterus it is necessary to modify the ionic equilibrium of the fluid medium. With the thiodiphenylamine derivatives (3277 RP) this antihistaminic action can be easily demonstrated since substances of this series have little action of their own on the guinea pig uterus.

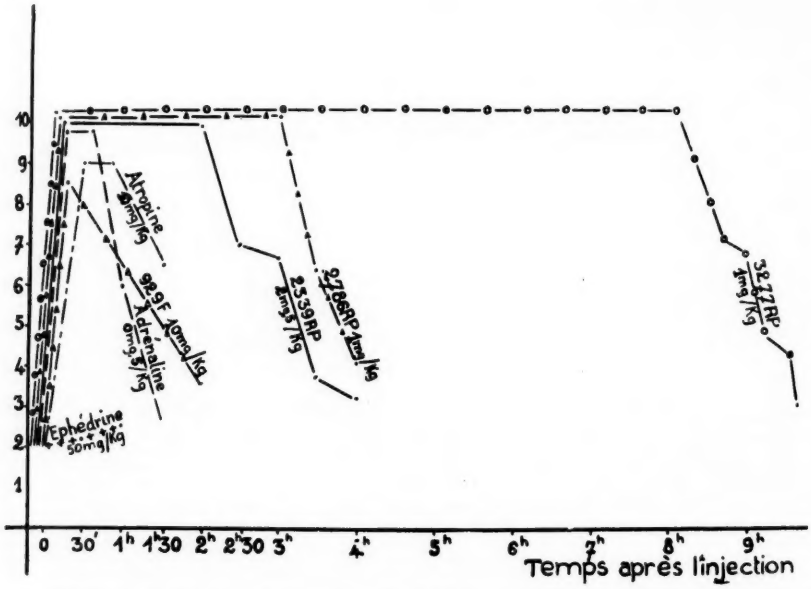


Fig. 2.—Comparison of the antiasthmatic activity of some antihistaminic derivatives and some alkaloids by the technique of histamine aerosols in the guinea pig.

5. *Antagonistic action against the vascular effects of histamine.*—The antihistamines of the Antergan series and thus derived from pyridine are able to counteract more or less effectively according to the species of animal and the dose injected, the circulatory disturbances produced by histamine.[1] The latest compounds studied, especially 3277 RP, completely abolish the vascular effects of from 0.1 to 1 mg. per kilogram of histamine injected intravenously in the unanesthetized or urethanized rabbit. With the dog and cat whose vascular systems, as is well known, are very sensitive to histamine, the thiodiphenylamine derivatives may, depending on the dosage and the experimental conditions, diminish or completely abolish the hypotensive effects of from 0.1 to 1 mg. per kilogram of histamine. This effect is demonstrable both prophylactically and therapeutically.

6. *Antagonistic effect on the gastric secretion of histamine.*—As for gastric secretion, the thiodiphenylamine derivatives behave just like the other antihistamines, that is to say, they do not appreciably alter the gastric secretory effect of histamine. We have also noted lack of action of the antihistamines

against the salivary and pancreatic secretions provoked by histamine. This failure of the antihistamines in the realm of external secretions is disturbing and has not yet been explained.

7. *Histamine-induced gastric ulcer and perforation.*—The striking disparity between the general antihistaminic activity of the thiodiphenylamine derivatives (which enables an animal to tolerate up to 1,500 lethal doses of histamine) and their utter ineffectiveness against the gastric secretions induced by histamine has provided an explanation for one particular phenomenon that we have observed: animals that have received several hundred lethal doses of histamine without manifesting any immediate serious disturbances die unexpectedly in the following twenty-four or forty-eight hours. Necropsy shows the cause of death to be a fulminating peritonitis resulting from gastric perforation. Above a certain dosage of histamine (200 to 300 lethal doses), the animals show one or several gastric ulcers, usually on the posterior aspect near the greater curvature, which in some cases go on to perforate. A pathologic and x-ray study of these lesions is now in progress, and we shall report the result in due time.[9] However, it seems wise to draw attention at present to this particular characteristic syndrome which can be reproduced with striking regularity.

ANTIANAPHYLACTIC ACTION OF THIODIPHENYLAMINE DERIVATIVES. RELATIONSHIPS WITH TISSUE EOSINOPHILIA

As we have repeatedly emphasized, the essential interest of the antihistamines lies in their antianaphylactic properties. Accordingly, we have attempted to determine for this series, as we have already done for other compounds, to what degree their antianaphylactic power is parallel to their antihistaminic activity.

It is in general relatively easy to evaluate quantitatively a pharmacodynamic property of a substance, either by comparison with another substance of the same series or by determining its activity with respect to a biologically antagonistic substance. This is not the case, however, when the problem is to determine the activity of a substance upon a syndrome as complex as anaphylactic shock, which, by its very nature, excludes the possibility of any absolute comparison. Our idea was that the determination of the minimum dose that protects the animal against anaphylactic shock could serve as a basis of comparison. We chose the guinea pig, which, in our opinion, provides the best criterion for tests of anaphylaxis. In order to avoid the equivocal interpretation of mild anaphylactic shock, where the measurement of the severity of the symptoms depends on the critical spirit of the investigators, we have adopted as a criterion for these tests a shock so severe that it progresses rapidly and inevitably to a fatal termination.

This condition was achieved by sensitizing the animals with two intraperitoneal injections of 0.5 c.c. of sheep serum, three days apart. After an interval of fifteen to twenty-one days, if one injects in the vein 0.1 c.c. of the foreign serum, there results a very severe anaphylactic shock with death in three to six minutes in 100 per cent of the cases. By means of this rather drastic test, we have at-

tempted to determine the degree of activity of new compounds. We have sought to establish the minimum dose which, injected subcutaneously beforehand, is likely to protect the animal against fatal anaphylactic shock. Thus, it is possible to standardize substances with respect to their antianaphylactic properties. The results of these assays are summarized in the following table.

TABLE III. COMPARISON OF THE ANTIANAPHYLACTIC ACTIVITY OF SEVERAL SYNTHETIC ANTIHISTAMINES

PRODUCT INJECTED	DOSE IN MG/KG INJECTED SUBCUTANEOUSLY	DOSE OF SHEEP SERUM INJECTED IN JUGULAR VEIN (C.C.)	NUMBER OF ANIMALS	RESULTS
(Controls)		0.1	10	100% died in less than 5 minutes
N'dimethyl N'amino ethyl N benzylaniline (2339 RP antergan)	10	0.1	5	No symptoms. All survived
	1	0.1	6	33% showed a mild shock. All survived
	0.5	0.1	6	Severe shock. 4 survived
	0.25	0.1	5	Very severe shock. All died
N'dimethyl N'amino ethyl N p methoxy a aminopyridine (2786 RP neoantergan)	10	0.1	5	No symptoms. All survived
	1	0.1	4	All showed mild shock. All survived
	0.5	0.1	6	4 died
3015 R.P.	10	0.1	4	No symptoms. 100% survival
	5	0.1	5	id.
	2	0.1	5	2 died. 3 mild shock. 60% survived
	1	0.1	5	All died
3277 R.P.	1	0.1	5	No symptoms. 100% survived
	0.5	0.1	5	id.
	0.25	0.1	6	33% showed mild shock. 100% survived
	0.1	0.1	5	Moderate shock. 100% survived

From this table, we may conclude that dimethylamino-2-propyl-1 thiodiphenylamine hydrochloride possesses in addition to its remarkable antihistaminic properties, an antianaphylactic activity greater than that of the antihistamines so far studied. This substance shows the same degree of activity in vitro, according to the Schultz-Dale experiment. If 3277 RP in a concentration of 10^{-7} is added to Ringer's solution in which is immersed a fragment of the excised gut of a guinea pig sensitized to egg albumin or to sheep serum, the tissue fails to react to the antigen. Likewise, the subcutaneous injection, in a sensitized guinea pig, one-half hour before excision of the intestine, of 0.5 mg. per kilogram of the thiodiphenylamine derivative, abolishes the specific sensitivity of the tissue.

We have observed another significant tissue phenomenon. In guinea pigs that have experienced a severe but not fatal anaphylactic shock due either to insufficient antigen or not enough antihistamine to protect them completely, there can be found twenty-four hours later a marked tissue eosinophilia in the lungs. All around the bronchioles are masses of eosinophiles, invading the columnar epithelium of the bronchi, often bursting into the lumen of the bronchi and even present in the intrabronchial exudate. This pulmonary eosinophilia

(peri- and intrabronchial, often considerable) is completely absent, or present only in a very equivocal manner in guinea pigs whose anaphylactic shock was aborted by preliminary injection of a sufficient quantity of antihistamine. The parallel relation between the inhibitory action of the antihistamines on anaphylactic shock and on the eosinophilia is worthy of emphasis.

COMMENT

The antihistamines of the thiodiphenylamine series have in certain respects special characteristics which merit attention. We have observed a very considerable discrepancy between the action of these substances on certain physiological functions, and their antihistaminic power. When we examine 3015 RP we see that on histamine provoked bronchospasm as well as on the isolated guinea pig gut, it is barely as active as Antergan, whereas it is capable of protecting a guinea pig against 400 lethal doses of histamine. On the other hand, its antianaphylactic power is 2 or 3 times less than that of Antergan. Substance 3277 RP forms an even more striking example of this discrepancy. While roughly equivalent to Antergan in its action on the intestine and the bronchi, its antihistaminic power is about 40 times greater than that of Antergan, and its antianaphylactic power 5 times greater. Furthermore, among the thiodiphenylamine products studied there are some (3283 RP for example) which are much less active than Antergan in the various individual tests, but nevertheless possess equal antihistaminic activity.

Thus, one is obliged to recognize that the antihistaminic shock action of this series of substances does not run parallel with their antihistaminic activity exhibited on the various particular functions and that some other factor than "antitoxic property" is present in addition to the sum of these properties. Perhaps this antitoxic power is not even specific. Further tests will enable this to be determined.

SUMMARY

1. We have presented the results of our experimental investigation of a new series of antihistamines, the thiodiphenylamine derivatives.

2. Among the substances studied, the ones showing especially marked antihistaminic and antianaphylactic activity were 3015 RP and above all 3277 RP. Thus 3277 RP is able to protect the guinea pig against 1,500 lethal doses of histamine, and 0.1 mg. per kilogram is enough to protect this animal against fatal anaphylactic shock.

3. This new chemical series is distinguished by being less toxic and more active than earlier ones.

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A NEW METHOD FOR QUANTITATIVE EVALUATION OF ACTIVITY OF ANTIHISTAMINIC SUBSTANCES AND ITS APPLICATION TO THE STUDY OF BETA-DIMETHYLAMINOETHYL BENZHYDRYL ETHER HYDROCHLORIDE (BENADRYL)*

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THE methods for determination of antihistaminic properties have gained in importance in recent years because of the search for new histamine antagonists in connection with treatment of allergic manifestations. An exhaustive review of the work done to date may be found in the papers of Code,[1] Mayer,[2] and Feinberg.[3, 4]

Most of the in vitro and in vivo experimental methods utilize the supression of the histamine action on smooth muscle. Rennick and co-workers[5] used the inhibiting action on whealing in rabbits as indication of antihistaminic activity. In this paper we are presenting a method which uses capillary permeability for quantitative determination of antihistaminic activity.

We have shown in a previous communication[6] that the neutralization of diphtheric toxin in the skin by circulating antitoxin can be used for quantitative estimation of the effect of different substances on the permeability of skin capillaries. All the theoretical considerations and experimental proof will be found in the above mentioned paper,[6] and will therefore not be repeated here. From this procedure the new method for quantitative estimation of antihistaminic activity was developed. This method and its application to the measurement of antihistamine activity of beta-dimethylaminoethyl benzhydryl ether hydrochloride (benadryl) will be described in detail.

MATERIALS AND METHODS

Diphtheric toxin 1,116 and antitoxin No. 874 (1,600 units per ml.) were kindly placed at our disposal by the New York City Department of Health Laboratories. Dimethylaminoethyl benzhydryl ether hydrochloride (benadryl) for intravenous injection was obtained through the courtesy of Parke, Davis & Co. Where not otherwise stated, the dilutions of toxin, antitoxin, and histamine phosphate were made in 0.85 per cent saline solution shortly before use. White rabbits weighing approximately 2,000 Gm. were used throughout. Intravenous injections were made into the marginal ear vein. Intracutaneous injections were made into the skin of the back not less than twenty-four hours after shaving the animals with an electric shaver. Only animals with thin, not irritated, and intact skin were used.

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Two animals of approximately the same weight are used for an experiment. One animal receives the substance under investigation parenterally or orally, the second animal serves as a control. Soon after (in our experiments, five minutes after intravenous injection or thirty minutes after oral administration of benadryl), both animals are injected with 1 ml. of diphtheric antitoxin 1:50 (i.e., 32 units) intravenously. Immediately afterward each animal is injected intracutaneously with two series of twofold serial dilutions of diphtheric toxin in a volume of 0.1 ml. The dilutions are ranging from 1:100 to 1:1,280.

In one series the toxin is diluted in a solution of histamine phosphate (0.1 mg. per ml.), in the other series toxin dilutions are made in saline. The results are read after forty-eight hours. As the endpoint of the titration is taken the maximum amount of toxin completely neutralized (no reaction in the skin). It was shown in a previous communication[6] that if the capillaries are made maximally permeable to antitoxin 16 to 20 times more toxin is neutralized in the test series than in the saline series. This result should always be obtained in the control animal. Any diminution of this 16:1-"histamine to saline" ratio in the experimental animal must be ascribed to antihistamine activity of the substance under investigation, and can be expressed as per cent inhibition of histamine action (see Table I). As an example of this procedure we present Table II.

TABLE I. RELATIONSHIP OF THE "HISTAMINE : SALINE" RATIO TO THE PER CENT INHIBITION OF HISTAMINE ACTIVITY

"HISTAMINE : SALINE" RATIO	INHIBITION OF HISTAMINE ACTION IN PER CENT
16 : 1	0
8 : 1	50
4 : 1	75
2 : 1	87.5
1 : 1	100

TABLE II. INHIBITION OF HISTAMINE ACTIVITY BY INTRAVENOUSLY OR ORALLY ADMINISTERED ANTIHISTAMINE SUBSTANCE

DIPHTHERIC TOXIN 0.1 ML.	RABBIT NO. 100 TOXIN DILUTIONS MADE IN		RABBIT NO. 98 TOXIN DILUTIONS MADE IN		RABBIT NO. 547 TOXIN DILUTIONS MADE IN	
	HISTAMINE 0.1 MG. PER ML.	SALINE	HISTAMINE 0.1 MG. PER ML.	SALINE	HISTAMINE 0.1 MG. PER ML.	SALINE
1 : 100	N	N	N	++++	++++	N
1 : 200	++++	++++	N	++++	++	N
1 : 400	+++	++++	N	++++	+	N
1 : 800	++	+++	+++	+++	0	++++
1 : 1,600	+	+++	+++	+++	0	++
1 : 3,200	+	++	++	++	0	+
1 : 6,400	0	+	+	+	0	+
1 : 12,800	0	0	0	0	0	0
"Histamine : Saline" Ratio	2 : 1		1 : 1		16 : 1	
Inhibition of histamine action in per cent	87.5		100		0	

Rabbit No. 100 received 7.5 mg./kg. of benadryl intravenously, followed after 5 minutes by intravenous injection of 1 ml. (1:50) diphtheric antitoxin. Immediately afterward the serial dilutions of toxin were injected intracutaneously. Rabbit No. 98 received 250 mg. of benadryl orally. Thirty minutes later diphtheric antitoxin and toxin were given as in rabbit No. 100. Rabbit No. 547 received no benadryl and served as a control.

N = necrosis; + – ++++ = degree of skin reaction; 0 = no reaction.

EXPERIMENTAL

The method described above was used for a more systematic investigation of antihistaminic activity of intravenously administered beta-dimethylaminoethyl benzhydryl ether hydrochloride (benadryl). For this experiment seventeen white rabbits weighing 1,800 to 2,500 Gm. each were used. Benadryl was given to ten animals, seven animals served as controls. The test animals received 7.5 mg./kg. of body weight of benadryl intravenously five minutes before the injection of antitoxin. Some of the animals showed slight tremor after the injection, but no convulsions were noticed with this dose of the substance. The results of this experiment are summarized in Table III. It will be seen from Table III that benadryl suppresses very effectively the action of histamine on capillaries of the skin. This inhibition ranges from 75 to 100 per cent.

TABLE III. ANTIHISTAMINIC ACTIVITY OF BENADRYL

RABBIT NO.	"HISTAMINE : SALINE RATIO	INHIBITION OF HISTAMINE ACTION IN PER CENT
97	4 : 1	75
79	4 : 1	75
76	4 : 1	75
302	4 : 1	75
85	2 : 1	87.5
100	2 : 1	87.5
249	2 : 1	87.5
323	2 : 1	87.5
232	1 : 1	100
99	1 : 1	100
	Controls	
527		
544	16 : 1	0
550	16 : 1	0
543	16 : 1	0
526	16 : 1	0
95	16 : 1	0
37	16 : 1	0

Rabbits Nos. 97, 79, 76, 302, 85, 100, 249, 323, 232, and 99 received 7.5 mg./kg. of body weight of benadryl intravenously, followed 5 minutes afterward by intravenous injection of diphtheric antitoxin (1 ml. 1:50, i.e. 32 A.U.) and intracutaneous injection of serial dilutions of diphtheric toxin in saline and histamine (0.1 mg. per 1 ml.), respectively. Rabbits Nos. 527, 544, 550, 543, 524, 95 and 37 were injected with antitoxin and toxin only; they did not receive any benadryl, and served as controls.

DISCUSSION

From the papers of Mayer[2] and Feinberg,[4] it may be seen that many different methods have been used for the determination of antihistaminic activity. It remains to be seen whether the ratio of antihistaminic activity as determined by these different methods will be the same for all antihistaminic substances. Until this question is decided, each new method will be a contribution to the pharmacology of antihistaminic substances. The new method, which we describe in this paper, is easy to perform and gives consistent quantitative results. We hope, therefore, that it will become a useful tool in the search for new antihistaminic substances.

SUMMARY

1. A method is described which enables a quantitative appraisal of antihistaminic activity from the suppression of the action of histamine on skin capillaries in the rabbit.

2. Benadryl in an intravenous dose of 7.5 mg./kg. of body weight was found to suppress the action of histamine (1:10,000) on skin capillaries of a rabbit from 75 to 100 per cent.

3. The usefulness of this method for the testing of histamine antagonists is discussed.

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IMMUNOLOGIC STUDIES IN PATIENTS WITH SUBACUTE BACTERIAL ENDOCARDITIS TREATED BY COMBINED PENICILLIN HEPARIN METHOD*

II. Report of Sensitivity to Heparin in Patient

Max Grolnick, M.D. and Leo Loewe, M.D., Brooklyn, N. Y.

IT WAS originally demonstrated by MacLean[1] in 1916 that an extract of dog liver contained a substance which retarded the coagulation of blood in vitro. In 1918 Howell and Holt[2] named this substance "heparin" because of its abundance in the liver. Further studies showed that this material could be obtained from various mammalian sources. Charles and Scott[3] demonstrated the presence of heparin in various tissues, such as liver, spleen, heart, blood, thymus, blood serum, lung, and muscle. The greatest amount was obtained from muscle, liver, and lung, and smaller amounts from heart, thymus, spleen, and blood. Furthermore, the yield from one mammalian source was larger than that from another, e.g., dog liver contained approximately twice as much heparin as did beef liver. Larger sources of supply were made available when Charles and Scott[4] prepared a purified barium salt of heparin, and Schmitz and Fischer[5] succeeded in obtaining a pure brucine salt of heparin. Best[6] stated that the greatest practical yield of heparin was derived from beef lung as the crystalline barium salt. Jacques, Waters, and Charles[7] demonstrated that heparin obtained from sheep, pig, beef, and dog showed a relative biologic potency of 1:2:5:10. They felt that heparin obtained from different tissues from one animal was the same, but that heparin obtained from different animal sources varied.

In a comprehensive review on heparin, Best[6] stated that Howell demonstrated the carbohydrate nature of heparin. Charles and Scott established the presence of nitrogen, and Jorpes the presence of sulfur. Jorpes advanced the theory that heparin was a chondroitin polysulfuric acid, containing glucosamine and a uronic acid.

The most significant biochemical property of heparin in its power to react with protein. Fischer[8] concluded that heparin combined with protein on the acid side of the isoelectric point. He demonstrated that compounds could be formed with casein, hemoglobin, liver proteins, and protamines. The reaction of heparin with protein might result in inactivation of the heparin if the protein used was able to compete with the clotting system of the heparin. Jacques[9] confirmed and extended the work of Fischer.

After extensive experimental and clinical trials with purified heparin by numerous investigators, among them Best,[10] Murray,[11,12] Jorpes, Holmgren, and Wilander,[13] Crafoord and Jorpes,[14] McClure and Lam,[15] the value of this

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drug as an efficient and safe anticoagulant was established. It has been used in vascular surgery, in the therapy of thrombophlebitis and phlebothrombosis, and in the therapy and prophylaxis of postoperative pulmonary embolism. More recently its application in the treatment of subacute bacterial endocarditis has been stressed by Friedman, Hamburger, and Katz,[16] Kelson and White,[17] MacLean, Meyer, and Griffith,[18] and Loewe and co-workers.[19, 20]

Such untoward effects as local hematoma, hematuria, cerebral hemorrhage, fever and gastrointestinal symptoms have been reported from the administration of heparin. McClure and Lam,[15] in a report on eight patients with postoperative pulmonary embolism under heparin therapy, stated that chills and fever occurred in the earlier patients, but no such effects were noted in the last 5 consecutive patients. Murray[12] did not mention any type of reaction in 440 patients who were given heparin postoperatively to prevent pulmonary embolism. He stated that heparin had been administered to several patients at intervals of several months without signs of "anaphylaxis." Crafoord and Jorpes[14] treated 325 postoperative patients prophylactically for an average of 5 to 10 days using 250 to 300 mg. heparin daily in four spaced intravenous injections. In three patients only slight rises in pulse rate and temperature were noted. In another series, 88 patients were treated without untoward effects. The authors stated also that in Sweden at least 30,000 doses of heparin in amounts from 50 to 100 mg. had been given intravenously without fatality or noteworthy drawback. In only one case were there symptoms which were of an "anaphylactic" nature. No further details were given regarding this one instance. DeTakats[21] described a test for the determination of heparin response or "sensitivity." A 10 mg. quantity was injected intravenously and a curve plotted from the results obtained by serial blood coagulation determinations. The author referred to "hyperreactors, showing phenomena of sensitization, such as flushing of the face, difficulty in breathing, sweating, and faintness." In a group of 87 patients so studied, eight reacted with an increase in coagulation time to seven and one-half minutes. Seven of the eight showed "flushing of the face, dyspnea, choking, constriction of the chest, and a feeling of faintness, characteristic of sensitization phenomena." The author then drew an analogy between these findings and the delay in clotting which is present in the anaphylactic state. Six of the eight patients with the "allergic" reaction had never received heparin previously. The author then asked whether sensitization could occur to the endogenous product inasmuch as heparin is normally secreted into the blood stream.

The report which follows is of interest because of immunologic findings not previously reported, in a patient receiving combined penicillin-heparin therapy for subacute bacterial endocarditis. This patient was one of a series being studied for evidence of acquired penicillin sensitivity.[22]

CASE REPORT

L. M., male, aged 50 years, was placed under treatment with intravenous penicillin on Feb. 4, 1944. Three days later heparin was added to the infusion in sufficient amount to give optimum blood coagulation levels. Approximately 100 to 150 mg. (10 to 15 c.c.) were

used daily. Therapy was temporarily discontinued on the sixteenth day of combined treatment (February 19) because of a chill and rise in temperature to 103.4° F. The total amount of heparin used in this period was 1,600 mg.

Treatment with both penicillin and heparin was resumed twelve days later and continued for twenty-eight days. In this cycle 3,900 mg. of heparin had been given. In order to rest the veins, penicillin treatment was resumed two days later (April 1) by the intramuscular route. Heparin was then administered subcutaneously on April 3, 5, and 7 in amounts of 200 mg. each. On April 8 there was a rise in temperature to 102.4° F. After another rest period of three days, penicillin and heparin were again given intravenously. Chill and fever ensued during the next twenty-four hours. Resumption of treatment one day later was again followed by chill and fever. Treatment was discontinued for seven days. Then, within an hour and one-half after administering penicillin and heparin intravenously (April 21), chill and fever again occurred. Erythema appeared over most of the body, especially on the face and neck. Scattered hives were present the next day, and recurred off and on for another day.

Consultation was requested on April 24 for opinion as to a possible allergic cause of the above noted symptoms. The patient gave a negative personal and family history of allergy. It was thought at first that penicillin might be the offending factor inasmuch as large amounts, from one-half to one million units daily were being given. Skin and ophthalmic tests with penicillin solution 5,000 and 10,000 units per c.c. were negative on several occasions. Intracutaneous tests with the mold extracts penicillium notatum, digitatum, and rubrum, and with alternaria and hormodendrum were negative. Tests however were positive with heparin solution in varying dilutions, as follows:

Heparin 1:10,000	0+	(neg. sl.)
1:1,000	±	(sl.–)
1:100	+	(sl.)
1:10	+± to ++	(sl. mod. to mod.)
stock	++ to ++±	(mod. to mod.+)

Skin tests were repeated on three subsequent occasions with comparable findings. Ophthalmic tests on three occasions gave negative reactions. Blood taken on May 4 was studied by the passive transfer method* with the following results:

Heparin 1:10 and stock	–	positive
Penicillin 5000 u/c.c.	–	0
Diluting Fluid	–	0
Beef Liver	–	0
Beef Extract	–	0

Precipitin tests did not reveal the presence of antibodies for penicillin or heparin.*

All treatment was stopped for three weeks. Penicillin alone was then administered intravenously, but, after a period of ten days, had to be discontinued because of recurring phlebitis at the venipuncture sites. Considering the gravity of the patient's condition, and since it was felt that his best chance for recovery was by the intravenous route employing both penicillin and the anticoagulant heparin, an attempt to hyposensitize the patient against heparin was undertaken.

Beginning on June 15 with a 1:1,000,000 dilution of heparin, small quantities were injected subcutaneously in increasing amounts at frequent intervals. By the second day a 1:100 solution was being given at intervals of six to twelve hours, with a total dose of 1.4 mg. heparin for the day. Small local reactions were produced, lasting up to an hour or more. Headache was complained of after some of the injections.

Stock solution of heparin was employed on the third day, the total for the day being 3.0 mg. On the next day 10.0 mg. was administered. Following a single injection of 35.0 mg. (larger than the prescribed dose) on the fifth day, a moderately large local reaction was produced. Scattered hives resulted on and off during the day. The dose was reduced

*Performed by Katherine L. Bowman.

and then gradually increased. A few small hives recurred after a dose of 30.0 mg. Subsequently a dose of 50.0 mg. at 6- to 12-hour intervals was tolerated without producing systemic symptoms. By the ninth day a total of 150.0 mg. was injected, and the route was changed from subcutaneous to intramuscular. On the eleventh day 15.0 mg. were administered intravenously without ill effect. During the next three days both intramuscular and intravenous routes were employed. On the fifteenth day 100.0 mg. of heparin were given intravenously together with the penicillin. On the seventeenth day (July 1) blood was taken for passive transfer studies. On the eighteenth day, heparin was omitted because of a mild febrile reaction. Two days later the dose was again built up from 25.0 mg. to 75.0 mg. daily. Combined therapy was thereafter continued until the thirtieth day of the hyposensitization procedure when a febrile reaction occurred again. Though the dose of heparin was reduced once more, fever continued and all therapy was stopped two days later (July 16).

After a rest period of eleven days, penicillin was started intravenously (July 27), and the next day 25.0 mg. of heparin were added to the infusion. The following amounts were given on successive days, 40.0, 55.0, and 70.0 mg. Several wheals then appeared and the temperature began to rise. On the next day (fifth) 85.0 mg. was included, and there was a febrile rise to 103.6° F. with marked urticaria. Heparin was omitted thereafter. Penicillin was then given either by intramuscular or intravenous routes, and sulfadiazine was administered by mouth. Blood culture became negative on August 17. The patient was skin-tested on August 17 and September 11. Treatment with penicillin was continued up to September 18. The blood remained sterile and the patient was discharged on Nov. 1, 1944.

Blood taken on July 1 for passive transfer tests was studied to determine whether the animal source of heparin was a factor in the allergic picture. Passively sensitized sites were tested with extracts of beef serum (1:10) and beef lung (1:10), in addition to heparin. Positive transfer reactions were obtained to all three preparations.

Direct tests done on August 17 and September 11 gave the following findings:

		8/17	9/11
Diluting Fluid	–	0 (neg.)	0 (neg.)
Heparin (1:10)	–	+ (sl.)	± (neg. sl.)
Heparin (stock)	–	++± (mod.+)	+± (sl. mod.)
Beef Serum (1:10)	–	+± (sl. ++)	++ (mod.)
Beef Lung (1:10)	–	+ (sl.)	+± (sl. mod.)
Cow Epith. (.01)	–	0 (neg.)	0 (neg.)

It was not possible to test the patient with other sera, animal tissues, and epidermal extracts, because of his refusal to cooperate further.

The patient was readmitted to the hospital on January 16, 1945, with a positive blood culture which had appeared after a severe upper respiratory tract infection. Penicillin therapy was started intravenously on January 18, but was stopped several days later because of a local phlebitis with elevation in temperature. When penicillin therapy was resumed (January 13), 10.0 mg. of heparin were added to the infusion, and the dosage was increased daily by the same amount until 100.0 mg. were being given (January 22). Blood was taken for passive transfer study on January 17. Two million units of penicillin and 100.0 mg. of heparin were being administered daily. On February 6 and again on February 12 there was a rise in temperature to 101° F. On February 21 the temperature rose again to 104.4° F. Headache was present, but no hives appeared. All therapy was discontinued then because blood culture had been negative since January 19. Penicillin therapy had been given in this series for a period of forty-four days for thirty-nine of which it had been combined with heparin. The patient was discharged on April 18.

No direct allergic studies could be carried out during this admission because the patient refused to cooperate. The specimen of blood taken for passive transfer study on January 7 (four days after heparin had been added to the treatment) did not reveal any

reactions to heparin, beef, cow epithelium, beef liver, pork, and horse epithelium. A small transfer to beef serum (1:10) was obtained, but was not confirmed on repetition of the test from the same serum specimen.

DISCUSSION

In interpreting the findings of fever, chill, and urticaria, four possible etiologic factors had to be considered, namely, penicillin, heparin, phlebitis, and the infection itself. The first cycle of treatment lasted sixteen days, terminating in chill and fever. After a rest period of twelve days further combined intravenous therapy was not attended by any untoward effects, indicating that the initial reaction was probably not an allergic manifestation. The next cycle of treatment consisted of several subcutaneous injections of both drugs at spaced intervals. Fever and chill resulted following the third injection of heparin in this course. Thereafter, two further attempts to include heparin in the intravenous infusion were promptly followed by chill and fever. Urticaria appeared with the last episode.

This series of events comprising several courses of treatment with intervening rest periods, eventually resulting in fever, chill, and urticaria, suggested acquired drug sensitivity of the serum disease type. Penicillin as the offender would seem to be ruled out by the fact that treatment with penicillin alone for short periods during the first hospital admission and for longer periods during the second stay was not attended with any reactions. The absence of positive direct tests and of reagins for penicillin also gave some support to this view, though such findings do not generally occur with drug hypersensitiveness. The presence of positive direct skin tests and of reagins to heparin favored consideration of the latter as the cause of the allergic manifestations. This was further substantiated when, during the hyposensitization attempt with heparin, urticaria occurred several times. The evidence would therefore seem to point to heparin as the cause of the reactions. The initial bout of chill and fever may have been due to the disease itself or to an underlying phlebitis.

The hyposensitization procedure consisted of a series of subcutaneous, then intramuscular injections during an eleven-day period, followed by the intravenous route. Heparin was then tolerated for twenty-two days, after which subsequent attempts to use it were again followed by chill, fever, and urticaria. Any explanation for this temporary tolerance for heparin would be theoretical.

During the second hospital stay heparin was given intravenously in increasing amounts beginning with 10 mg., up to the indicated daily dose of 100 mg. The first temperature rise occurred on the twenty-fourth day of such treatment. Here again, there had been a temporary tolerance to the administration of heparin with eventual development of the allergic state. The passive transfer study of blood taken four days after heparin therapy was reinstituted showed an absence of reagins to heparin and beef lung and a questionable one to beef serum. It would seem that there had been insufficient contact with the drug up to this point to restimulate reagin production.

It must now be considered whether the findings indicate an acquired drug allergy to heparin or to an associated product from the animal source of the

drug. In the case described, reagins to heparin, beef serum, and beef lung were obtained. Uncompleted studies on the relationship of heparin to its source now being carried out on normal subjects, on atopic patients, and other patients who received combined penicillin-heparin therapy for subacute bacterial endocarditis would seem to point to beef serum as the offending factor. These findings will be the subject for a further communication.

SUMMARY

A case of induced sensitivity to the drug heparin is reported. Reagins were demonstrated to heparin, beef serum, and to beef lung which is the source of heparin.

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Book Review

Allergy in Theory and Practice. Robert A. Cooke, M.D., Philadelphia, 1947, W. B. Saunders Co., pp. 572, illustrated.

This book was written in collaboration with thirteen associates, many of whom are distinguished in their respective interests in Allergy and Immunology and include such authorities as Michael Heidelberger, Paul Klemperer, and Louis Schwartz. It is therefore perhaps the most authoritative of the dozens of volumes on the subject that have been published recently. It is comprehensive, beautifully illustrated, well organized, and well documented with relevant references.

There is little doubt that if one conceives of Allergy and practices it according to this book, he will deal with the subject on a high level. Allergy is very complex, however, and conceptions of mechanisms, classifications, and techniques are by no means crystallized. This book presents opinions of the individual authors and, although some discussion of other points of view does appear, the reader is not left much choice. This does not detract from its content, but more discussion here and there of the viewpoint of others would correct, in the opinion of this reviewer, its one fault.

Correspondence

New York, May 7, 1947.

To the Editor:

The following is an abstract of an article that appeared in the Dec. 13, 1946, issue of *Science* which I believe will be of interest to the membership of the Academy.

NEW HORIZONS IN MEDICAL RESEARCH

C. J. Van Slyke, M.D.

Medical Director, U. S. Public Health Service, and Chief, Research Grants Division
National Institute of Health, Bethesda, Maryland

Dr. Van Slyke described the United States Public Health Service Research Grants Program which is characterized by a sincere and continuing effort to supply Federal funds for the support of necessary research in the various fields of medicine and related sciences.

This program is wholly scientific, under the guidance of scientists, and no degree of government restriction, control or supervision is interposed. Study sections in various specialties of medicine have been developed. Grants in aid have been authorized for medical research to universities, hospitals and individuals.

Applications for grants in aid for medical research should be made on application forms obtainable from and later submitted to the Chief, Research Grants Division, National Institute of Health, Bethesda 14, Maryland. Anyone interested is advised to read Dr. Van Slyke's article in the issue of "Science" above mentioned.

Yours sincerely,

Will C. Spain, M.D.

American Academy of Allergy

NEWS AND NOTES

REPORT OF THE NATIONAL POLLEN SURVEY COMMITTEE OF THE AMERICAN ACADEMY OF ALLERGY ON A CONVERSION FACTOR FOR GRAVITY SLIDE COUNTS

The recommendations on standardization of pollen counting techniques made in an earlier report by this Committee (J. ALLERGY **17:** 178, 1946) have been favorably received and are being generally adopted. The standard slide exposure instrument is rapidly replacing the miscellaneous devices previously employed and pollen counts are now being reported in terms of granules per square centimeter of 24-hour slide.

This Committee has investigated the advisability of standardizing the method of converting the gravity slide figures to some volumetric equivalent and has arrived at the following conclusions:

1. It is the opinion of this Committee, as it has been of most aerobiologists since the time of Blackley and Scheppegrell, that a *volumetric* measure would be the preferred basis on which to report the pollen content of the air.

2. There is no *simple* volumetric device now available for directly measuring the pollen content of the air which could be readily applied on a nationwide scale. Under the circumstances, the gravity method, although subject to many limitations, provides the only practical counting procedure which can be universally employed and must therefore be retained.

3. Because of uncontrollable air current factors, as well as other variables which apply, to all local slide exposure stations, conversion factors based solely upon theoretical rates of fall of pollen grains and computed by applications of Stokes law or modifications thereof are unsatisfactory.

4. For the present, a conversion factor based upon actual comparative determinations made with the standard gravity sampling technique and a reliable volumetric sampler represents the only practical approach to the solution of this problem.

A series of comparative volumetric gravity samplings made by Durham in Chicago and Milwaukee in the ragweed season of 1945 (J. ALLERGY **17:** 82, 1946) revealed that the *average catch of ragweed pollen on one square centimeter of slide exposed in the standard instrument compared with the average catch per cubic yard of air by a simultaneously operated and independently calibrated volumetric sampler was as 1 to 3.6.* This Committee therefore recomends that 3.6 be employed as the standard factor for the conversion of ragweed pollen counts from gravity slide figures (1 square centimeter of 24-hour slide) to cubic yard equivalents.

It is further recommended that in publications of a technical nature, the slide area counted (1 square centimeter) and the conversion factor (3.6), if employed, be clearly stated. When figures are released for the press and for nontechnical purposes, the square centimeter reference may be omitted and the count presented as "the number of ragweed pollen grains per cubic yard of air as determined by the standard technique recommended by the National Pollen Survey Committee of the American Academy of Allergy."

The recommendations of this Committee on the 3.6 factor were submitted for consideration to the Advisory Council of the Section of Aeroallergens of the American Academy of Allergy. They were unanimously approved by the Council membership consisting of H. Black, R. A. Cooke, O. C. Durham, S. Feinberg, S. Hooker, Chairman, F. Simon, H. Stevens, and M. Walzer. In lieu of voting, E. Stakman, who was abroad, sumitted the proposals to three members of the Committee on Aerobiology of the National Research Council for their consideration. Of these S. Mudd and E. Lambert approved, and R. Wodehouse disapproved.

With this almost unanimous endorsement, this Committee submits the 3.6 factor for the volumetric conversion of ragweed gravity slide counts obtained with the standard technique. Conversion factors for other pollens have not yet been approved by this Committee but are under consideration.

O. C. DURHAM, Technical Director
J. H. BLACK
J. GLASER
M. WALZER, Chairman

COMMITTEE ON STANDARDIZATION

A subcommittee of the Committee on Standardization met in New York and planned an outline by which commercial firms could prepare extracts. After these extracts have been prepared, neutralization tests to check on their potency will be performed. Members of the committee interested in this problem will please contact the chairman, Dr. Chobot, at once so that he may arrange their participation in this activity. It is hoped that as a result of this decision, progress will be made as to the uniformity of protein extracts and that at least ragweed and other antigens will be standardized in this fashion.

Dr. Arthur Stull has consented to act as a member of this subcommittee and all procedures of preparation will be standardized according to his directions. Should there be any comments or suggestions that members of the committee have to make, please communicate with the chairman.

WASHINGTON AND BALTIMORE ALLERGISTS

Washington and Baltimore Allergists held a joint meeting April 23 at the Army-Navy Club in Washington. Earlier in the year the first meeting was held in Baltimore. Dr. Harry S. Bernton of Washington introduced the idea of such a meeting. Dr. Leslie Gay of Baltimore is Chairman of the Program Committee.

SOUTHWEST ALLERGY FORUM

At the recent meeting of the Southwest Allergy Forum held in Shreveport, La., Doctor Herbert Rinkel of Kansas City, Mo., was elected President. The President-elect is Doctor Sims Hulsey of Fort Worth, Texas. The next meeting of the Forum will be held in Oklahoma City, Oklahoma, in the spring of 1948.

LOUISIANA STATE ALLERGY SOCIETY

A group practicing Allergy in Louisiana have formed the Louisiana State Allergy Society. This Society will meet concurrently with the Louisiana State Medical Society. The officers were elected as follows: *President*, Doctor B. G. Efron, New Orleans, La.; *Vice-President*, Doctor Henry D. Ogden, New Orleans, La.; *Secretary*, Doctor Dudley Youman, Shreveport, La.

LOS ANGELES COUNTY MEDICAL ASSOCIATION

The first program of the section of Allergy of the Los Angeles County Medical Association on March 28, 1947, was as follows: (1) "Physiological Mechanism of Bronchial Asthma," Doctor Hyman Miller. (2) "Induced Allergy," Doctor Frank G. Crandall, Jr. At the April meeting the subject was "Immuno-Chemical Aspects of Allergy," Doctor Dan H. Campbell, Professor of Immuno-Chemistry, California Institute of Technology.

ASSOCIATED ALLERGY CLINICS OF GREATER NEW YORK

The Associated Allergy Clinics of Greater New York held its spring meeting April 15 at the Flower-Fifth Avenue Hospital. Speakers included Doctor Harold Abramson and Doctor Sheppard Siegel of Mt. Sinai Hospital, Doctor Murray Albert of Greenpoint Hospital, and Doctor Lawrence Farmer of Lenox Hill Hospital. This association was organized in 1937 by Doctor Robert Chobot, and now consists of 32 clinics with a membership of over 200 members of various allergy clinics in the five boroughs.

PHI LAMBDA KAPPA AWARD

Doctor Philip Levine last January received the annual scientific award of Phi Lambda Kappa, national medical fraternity. He was one of the three men credited with the discovery of the Rh factor in blood, and is currently director of the Ortho Research Foundation, Linden, New Jersey.

DIRECTORY OF AMERICAN ACADEMY OF ALLERGY

Any Member or Fellow of the Academy who fails to receive his copy of the Directory of the American Academy of Allergy should commmunicate at once with Dr. Theodore L. Squier, Secretary of the Academy, 425 East Wisconsin Avenue, Milwaukee 2, Wisconsin.